



Biological Assessment Report

**Douger Branch/Chat Creek
Lawrence County, Missouri**

Fall 2019 - Spring 2021

Prepared for:
Missouri Department of Natural Resources
Division of Environmental Quality
Water Protection Program
Water Pollution Control Branch

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1.0 Introduction

At the request of the Missouri Department of Natural Resources (**MoDNR**) Water Protection Program (**WPP**), the Environmental Services Program (**ESP**) Water Quality Monitoring Section (**WQMS**), conducted a macroinvertebrate biological assessment and habitat study of Douger Branch/Chat Creek in Lawrence County, Missouri. United States Geological Survey (**USGS**) topographic maps delineate the downstream portion of this stream as Douger Branch and the upper portion as Chat Creek, separated by an unnamed tributary. Due to the separate names on topographic maps, this stream has two separate Water Body Identification numbers (**WBID**) in the Missouri Water Quality Standards (**WQS**, MoDNR 2018f); Douger Branch is designated as WBID 3810 and Chat Creek is designated as WBID 3168. Both WBIDs are listed in the WQS (MoDNR 2018f) as class “C” streams with the following designated uses: irrigation, livestock and wildlife protection, protection of warm water habitat and human health protection, category “B” whole body contact recreation, and secondary contact recreation. A class “C” stream is defined as a stream that may cease flow during drought periods but maintains permanent pools which support aquatic life. Category “B” whole body contact applies to waters that are designated for whole body contact recreation but are not considered public use areas.

Both WBIDs were on the 2018 303(d) List of Impaired Waters. Chat Creek, WBID 3168, was listed for cadmium contamination in the water resulting from the Baldwin Park Mine in Aurora, Missouri. In 2020 the U.S. Environmental Protection Agency (**EPA**) approved removal of Chat Creek from the 2020 303(d) List of Impaired Waters because the equation for calculating cadmium criteria changed, and cadmium levels now meet the Water Quality Standards. Douger Branch, WBID 3810, was listed on the 2018 303(d) List of Impaired Waters for lead and zinc contamination in the sediment resulting from the Aurora Lead Mining District. The equations for calculating lead and zinc contamination have not changed; therefore, Douger Branch remains on the 2020 303(d) List of Impaired Waters. A Total Maximum Daily Load (**TMDL**) study to address zinc was approved in 2006 for Douger Branch (MoDNR 2006). The Aurora Waste Water Treatment Facility (**WWTF**) discharges to Chat Creek, WBID 3168 and has daily maximum and monthly average limits for total recoverable zinc. Under permit #MO-0036757, the facility is required to submit monitoring reports quarterly.

Douger Branch/Chat Creek is classified as a losing stream. A losing stream is a stream that distributes 30 percent or more of its flow through natural processes such as through permeable geologic materials into a bedrock aquifer (MoDNR 2018f). The outfall for the Aurora WWTF is approximately 0.3 miles downstream of Chat Creek station 1.1 and 1.5 miles upstream of Douger Branch station 1.9. This facility is classified as a major municipal discharge of domestic wastewater with a design flow of 2.0 million gallons per day.

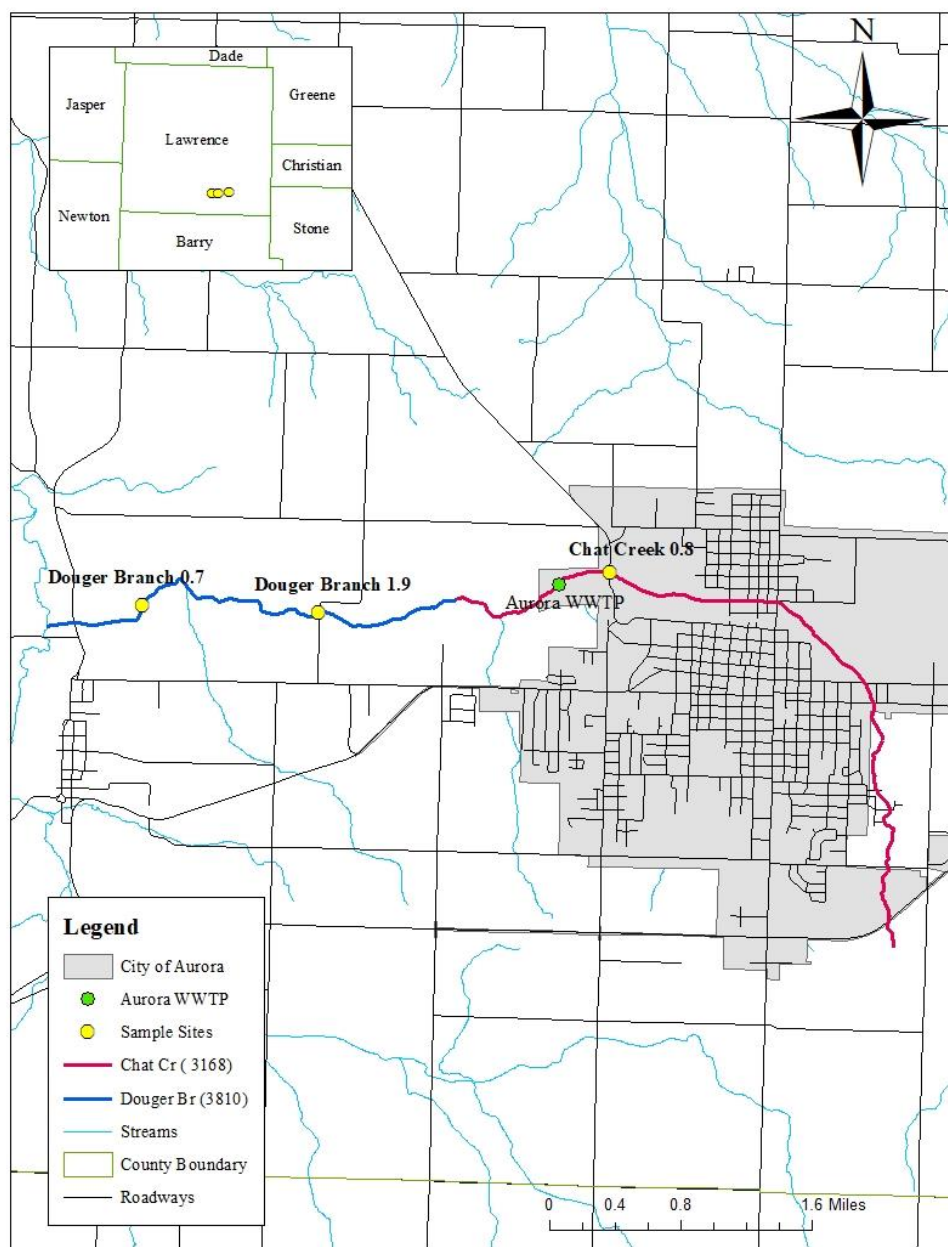
The goal of this study was to assess water quality based on stream habitat, water chemistry, and macroinvertebrate communities in Douger Branch/Chat Creek. Three stations were selected for this study: two stations on Douger Branch, WBID 3810, and one station on Chat Creek, WBID 3168. Bioassessment sampling was scheduled to occur during fall 2019 and spring 2020. Due to severe drought conditions during fall 2019, only Douger Branch 1.9 had sufficient water to sample. Douger Branch 0.7 and Chat Creek 1.1 had small isolated pools and lacked flowing

water and were therefore not sampled. In March of 2020, MoDNR staff were instructed to telework and avoid traveling due to the global COVID-19 pandemic, which resulted in spring sampling for this study being postponed until spring 2021. During the spring of 2021, all three stations were sampled: Douger Branch 1.9, Douger Branch 0.7, and Chat Creek 1.1. Bioassessment sampling included macroinvertebrate and water quality samples. Benthic sediment samples were collected in May 2020 after limited travel was reinstated. Habitat assessments were conducted during the summer of 2021.

2.0 Study Area

Douger Branch/Chat Creek is located in the Ozark/Neosho Ecological Drainage Unit (**EDU**; Figure 1). An EDU is a region in which biological communities and habitat conditions can be expected to be similar. Douger Branch/Chat Creek was assessed for this study using two sets of biological criteria. Macroinvertebrate samples for all sites were first compared to Ozark/Neosho EDU wadeable/perennial biological criteria reference (**BIOREF**) streams. Second, macroinvertebrates were compared to headwater candidate reference stream (**HWREF**) criteria calculated from stream stations similar in size to Douger Branch/Chat Creek to address potential macroinvertebrate community effects due to stream size. Three of the HWREF streams were chosen for comparison of habitat assessments, water chemistry, and benthic sediment data. HWREF streams were selected using an ArcGIS model developed at the University of Missouri that predicts the highest quality streams for creeks and small rivers (Kleekamp 2016). An analysis of the selected HWREF streams was also conducted using the 13-step selection process described in the *Selecting Small Candidate Reference Streams* section of the 2020 Listing Methodology Document (MoDNR 2019a). Each of the HWREF streams met the necessary conditions, and all were used to calculate small stream biological criteria in this report.

Figure 1. Douger Branch/Chat Creek Sampling Locations



2.1 Station Locations and Descriptions

All Douger Branch/Chat Creek sampling stations were in Lawrence County, Missouri. Station locations and descriptions are listed below and shown in Figure 1. All study stations and HWREF stations were in geographic Universal Transverse Mercator (UTM) zone 15. See Figure 2 for locations of HWREF in relation to Douger Branch/Chat Creek.

Sampling locations of Douger Branch/Chat Creek

Douger Branch 0.7 (NW ¼ sec. 9, T. 26 N., R. 26 W.) was the most downstream station on Douger Branch/Chat Creek and was located 0.5 miles upstream of the Highway P bridge. Geographic coordinates of the downstream terminus of the sampling reach are UTME 429910, UTMN 4092587.

Douger Branch 1.9 (SW ¼ sec. 10, T. 26 N., R. 26 W.) was located downstream of County Road 1165. Geographic coordinates of the upstream terminus of the sampling reach are UTME 431631, UTMN 4092507.

Chat Creek 1.1 (NW ¼ sec. 12, T. 26 N., R. 26 W.) was located upstream of the Carnation Road/Hwy 39 bridge. Geographic coordinates of the downstream terminus of the sampling reach are UTME 434487, UTMN 4092910.

Sampling locations of HWREF streams

Macroinvertebrate sampling from the following HWREF sampling locations were used in calculating HWREF stream criteria. Three stations, Dry Fork 2.3, Tributary to Cedar Creek 0.2, and Tributary to Cedar Creek 0.3 were also used for comparison in habitat assessments, water chemistry analysis, and benthic sediment analysis.

Dry Fork 2.3 (SE ¼ sec. 32, T. 29 N., R. 27 W.) is a HWREF stream located within the Talbot Conservation Area, located south of Hwy 96 and north of County Road 2075 in Lawrence County. Geographic coordinates of the upstream terminus of the sampling reach are UTME 420599, UTMN 4115064.

Tributary to Cedar Creek 0.2 (NE ¼ NW ¼ NW ¼ sec. 28, T. 26 N., R. 32 W.) is a HWREF stream located upstream of Trout Farm Road in Newton County. Geographic coordinates of the downstream terminus of the sampling reach are UTME 371815, UTMN 4090498.

Tributary to Cedar Creek 0.3 (SE ¼ NW ¼ NW ¼ sec. 28, T. 26 N., R. 32 W.) is a HWREF stream located upstream of Trout Farm Road in Newton County. Geographic coordinates of the midpoint of the sampling reach are UTME 371680, UTMN 4090236.

Pottershop Hollow 0.4 (NW ¼ sec. 04 T. 23 N., R. 31 W.) is a HWREF stream located upstream of Quail Road in McDonald County. Geographic coordinates of the downstream terminus are UTME 385226, UTMN 4067940.

Tributary to Cedar Creek 1.5 (NW ¼ sec. 33, T. 26 N., R. 32 W.) is a HWREF stream located downstream of Holly Road in Newton County. Geographic coordinates of the upstream terminus are UTME 371463, UTMN 4088642.

Tributary to Glendale Fork 3.0 (NE ¼ sec. 09, T. 31 N., R. 33 W.) is a HWREF stream located upstream of State Highway M in Shawnee Trail Conservation Area, Barton County. Geographic Coordinates of the downstream terminus are UTME 362473, UTMN 4147162.

Tributary to Glendale Fork 3.3 (NE ¼ sec. 09, T. 31 N., R. 33 W.) is a HWREF stream located downstream of SW 30th Road in Shawnee Trail Conservation Area, Barton County. Geographic coordinates of the upstream terminus are UTME 362251, UTMN, 4147420.

Tributary to North Fork Spring River 1.0 (SW ¼ sec. 33, T. 32 N., R. 29 W.) is a HWREF stream located near SE 90th Lane in Barton County. Geographic coordinates of the downstream terminus are UTME 399944, UTMN 4147937.

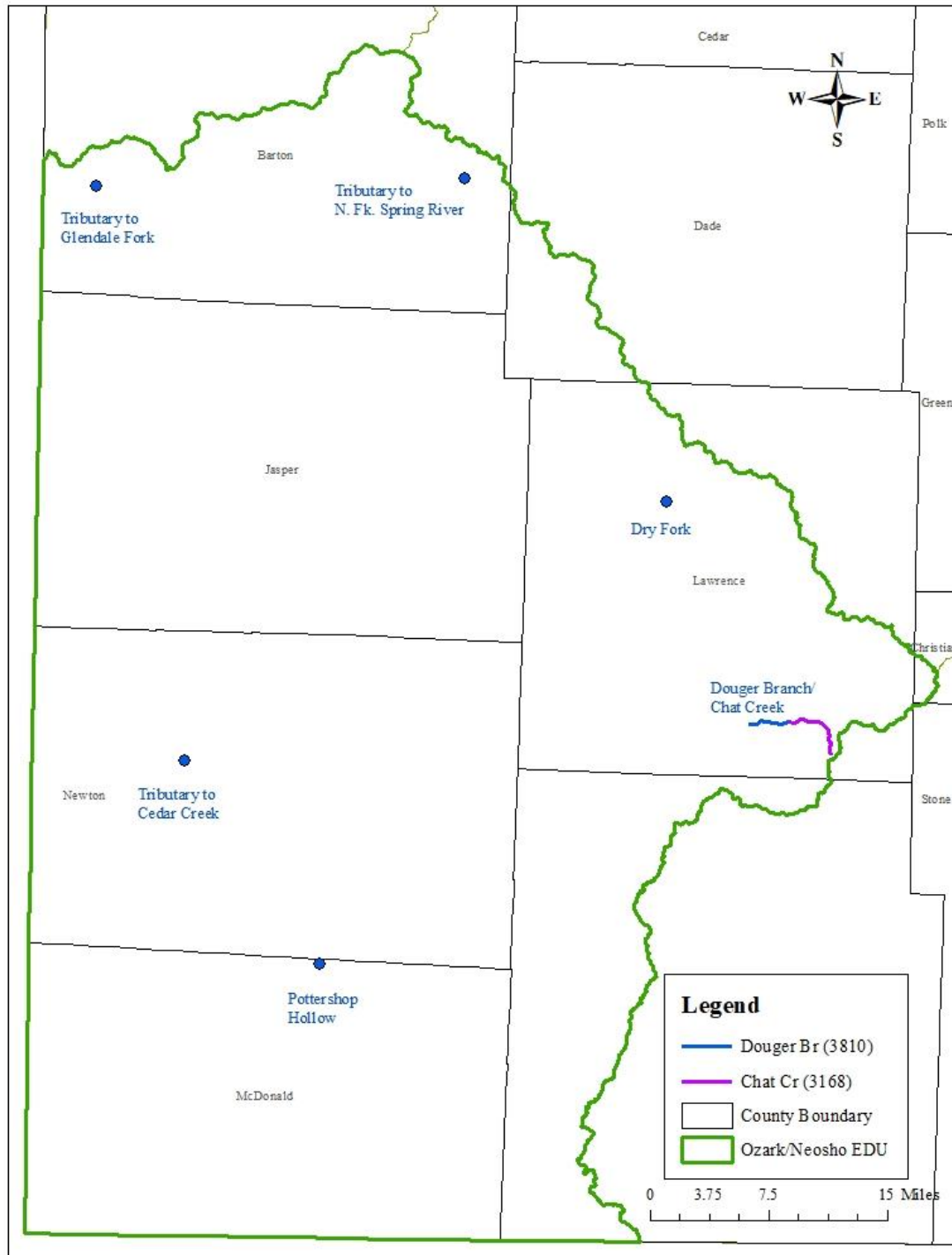
3.0 Objectives

The objective of this study was to determine if Douger Branch/Chat Creek supports its protection of warm water habitat beneficial use designation. The macroinvertebrate community and the instream habitat were compared longitudinally among study stations as well as with BIOREF stream data and HWREF stream data from the Ozark/Neosho EDU. Water quality, nutrient parameters and benthic sediment toxicity data were compared longitudinally among study sites and with HWREF stations. Water quality parameters were compared with Missouri's WQS (MoDNR 2018f) and EPA's ambient water quality criteria recommendations for nutrients (USEPA 2000). Benthic sediment toxicity was compared with MacDonald's recommended Probable Effects Concentrations (**PECs**) (MacDonald et al. 2000) for metals toxicity and Probable Effects Quotient (**PEQ**) (MacDonald 2009 et al.).

3.1 Null Hypotheses

- 1) Land cover characteristics in the Douger Branch/Chat Creek watershed will be similar to land cover characteristics of the Ozark/Neosho EDU.
- 2) Stream habitat assessment scores of Douger Branch/Chat Creek will be similar to the average stream habitat scores of the HWREF streams located within the Ozark/Neosho EDU.
- 3) The macroinvertebrate community in Douger Branch/Chat Creek will be similar among longitudinally separate reaches of Douger Branch/Chat Creek.
- 4) The macroinvertebrate community in Douger Branch/Chat Creek will be similar to the macroinvertebrate community of BIOREF streams in the Ozark/Neosho EDU.
- 5) The macroinvertebrate community in Douger Branch/Chat Creek will be similar to the macroinvertebrate community of HWREF streams in the Ozark/Neosho EDU.
- 6) Physicochemical water quality of the study stream will meet the WQS and U.S. EPA's recommended values.
- 7) Metals toxicity values of benthic sediment samples collected from Douger Branch/Chat Creek will meet recommended PEC values calculated by MacDonald et al. (2000), and the values will be below the PEQ threshold.

Figure 2. Location of Douger Branch/Chat Creek and Headwater Reference Streams in the Ozark/Neosho EDU



4.0 Methods

Macroinvertebrate and water quality sampling of the study stations was conducted during fall 2019 and spring 2021 by WQMS's Aquatic Bioassessment Unit. Fall 2019 sampling of Douger Branch 1.9 was conducted on September 24. Douger Branch 0.7 and Chat Creek 1.1 lacked sufficient flow to adequately sample during the fall bioassessment sampling period. The three HWREF streams used for comparison of water chemistry data were sampled on September 25, 2019. Due to the pandemic, spring sampling was postponed from spring 2020 to spring 2021. All Douger Branch/Chat Creek stations were sampled on March 22, 2021. The three HWREF streams used for comparison of water chemistry data were sampled on March 29, 2021. The remaining HWREF streams used for development of comparable biological criteria were sampled over a four year period between fall 2017 and spring 2021.

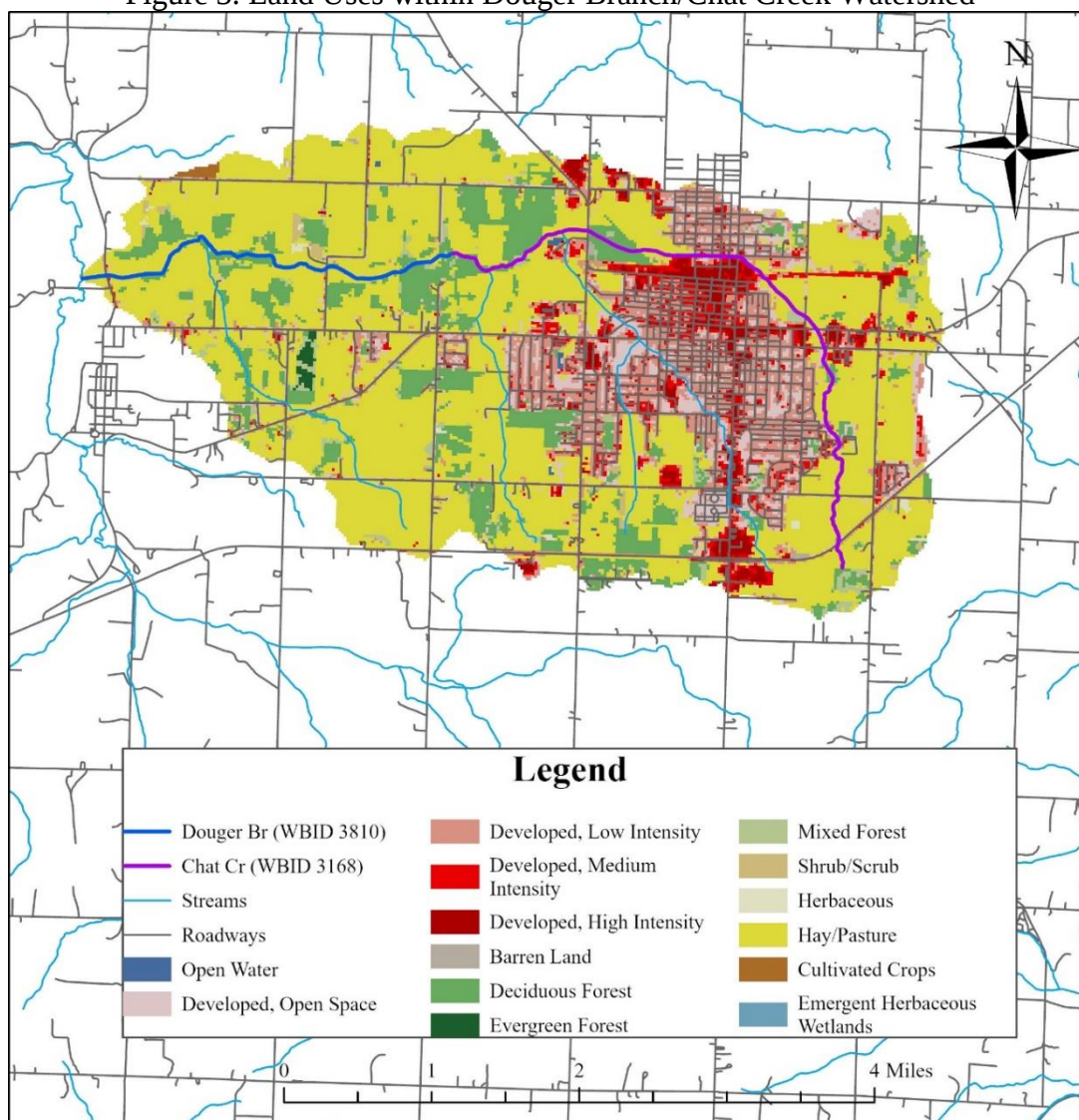
Benthic sediment samples were collected from Douger Branch/Chat Creek stations on May 29, 2020, and on three HWREF streams on May 18, 2020. Habitat assessments were postponed from fall 2019 until fall 2021 because two of the study stations, Douger Branch 0.7 and Chat Creek 1.1, consisted of small isolated pools and lacked flowing water. Chat Creek 1.1 lacked flowing water through riffles during fall 2021, which is reflected in the habitat assessment.

Methods for biological assessments, physicochemical water quality collection, habitat assessments, and benthic sediment sampling are included in this section. All samples were handled according to the appropriate MoDNR ESP Standard Operating Procedures (**SOP**) or Project Procedures (**PP**).

4.1 Land Use Description

Figure 3 shows the land use percentages of the Douger Branch/Chat Creek watershed containing the project sample reaches. Percent land use data were derived from the 2019 USGS national land cover database (USGS 2019) <https://www.usgs.gov/centers/eros/science/national-land-cover-database>)).

Figure 3. Land Uses within Douger Branch/Chat Creek Watershed



4.2 Stream Habitat Assessment Project Procedure

Standardized assessment procedures were followed as described for riffle/pool prevalent streams in the Stream Habitat Assessment Project Procedure (**SHAPP**, MoDNR 2019j). According to the SHAPP, an aquatic community is influenced by the quality of the stream habitat. Stream habitat quality is scored for each station, and the scores are compared with a control stream SHAPP score. If the SHAPP score at a test station is ≥ 75 percent of the SHAPP control score, the stream habitat at the test station is considered capable of fully supporting a biological community comparable to the control stream. If the test station score is 74 percent to 60 percent of the SHAPP control score, the stream habitat at the test station is considered capable of partially supporting a comparable biological community. If the SHAPP score at a test station is < 60 percent of the SHAPP control score, the habitat is considered non-supporting of the biological community.

4.3 Bioassessment

4.3.1 Macroinvertebrate Sampling and Analysis

Macroinvertebrate sampling was conducted according to the Semi-quantitative Macroinvertebrate Stream Bioassessment Project Procedure (**SMSBPP**, MoDNR 2019c). Douger Branch/Chat Creek is considered a riffle/pool dominated system. The three standard habitats sampled at all locations were flowing water over coarse substrate, non-flowing water over depositional substrate, and submerged root mat. Macroinvertebrate samples were subsampled in the laboratory and identified to taxonomic levels as specified in MoDNR's *Taxonomic Levels for Macroinvertebrate Identifications* SOP MDNR-ESP-209 (MoDNR 2019g) to develop biological metrics (MoDNR 2019c). Bench sheet data containing the macroinvertebrate taxa list for each study station are included in Appendix B. Macroinvertebrate bench sheet data for the HWREF streams are available upon request.

Douger Branch/Chat Creek macroinvertebrate data were evaluated relative to BIOREF streams in the Ozark/Neosho EDU. Biological criteria are calculated separately for the fall (mid-September through mid-October) and spring (mid-March through mid-April) index periods. The SMSBPP provides details on the calculation of metrics and scoring of the multi-metric Macroinvertebrate Stream Condition Index (**MSCI**). The four components of the MSCI are Taxa Richness (**TR**); Ephemeroptera, Plecoptera, and Trichoptera Taxa (**EPTT**); Biotic Index (**BI**); and the Shannon Diversity Index (**SDI**). An MSCI score of 16-20 is considered fully supporting, 10-14 partially supporting, and 4-8 non-supporting of the protection of warm water aquatic habitat beneficial use designation as listed in the Missouri WQS (MoDNR 2018f).

Douger Branch/Chat Creek samples were also compared to HWREF streams. This analysis was conducted by calculating biological metric threshold values using HWREF macroinvertebrate data, which were then used as biological criteria for the same four metrics listed above. This analysis was conducted to determine the extent to which stream size affected the macroinvertebrate community.

4.3.2 Physicochemical Water Sampling and Analysis

Physicochemical water samples were handled according to the appropriate MoDNR ESP SOPs. Results for physicochemical water parameters were examined by season and station by *in-situ* field measurements and grab samples collected in accordance with SOP MDNR-ESP-001, *Recommended Containers, Volumes, Preservatives, Holding Times, and Special Sampling Considerations* (MoDNR 2019d). All samples were kept on ice during transport to ESP.

Water quality parameters were measured *in-situ* or collected and returned for analyses at the ESP laboratory in Jefferson City. Turbidity (NTU, MoDNR 2018b) was measured in the ESP, WQMS biology laboratory. Discharge (cubic feet per second-cfs, MoDNR 2019f), dissolved oxygen (mg/L, MoDNR 2019e), conductivity (μ S/cm, MoDNR 2018e), pH (standard units, MoDNR 2018c), and temperature ($^{\circ}$ C, MoDNR 2018d), were measured in the field. The ESP Chemical Analysis Section conducted analyses for the following: nitrate+nitrite as N, total nitrogen, total phosphorus, ammonia as N, sulfate, chloride, total suspended solids/non-filterable residue, dissolved calcium, and dissolved magnesium (all parameters reported in mg/L). The ESP

Chemical Analysis Section also conducted analyses for the following dissolved metals (reported in µg/L): barium, cadmium, lead, nickel, and zinc.

Physicochemical water parameters were compared among stations as well as with Missouri's WQS (MoDNR 2018f). Nutrient values were compared to U.S. EPA's recommended nutrient values for rivers and streams in nutrient ecoregion XI (USEPA 2000). Interpretation of acceptable limits in the WQS may be dependent on a stream's classification and beneficial uses as designated in the WQS (MoDNR 2018f).

Benthic sediment was collected in accordance with SOP MDNR-ESP-011, *General Sampling Considerations for Chemical Analysis of Sediment* (MoDNR 2018a). The ESP Chemical Analysis Section conducted analyses of the benthic sediment samples for the following metals (reported in µg/kg): arsenic, cadmium, chromium copper, lead, nickel, and zinc. Analysis of total organic carbon in benthic sediment samples was conducted by a contract laboratory.

4.3.3 Discharge

Stream flow was measured using a SonTek/YSI FlowTracker® Handheld Acoustic Doppler Velocimeter® at all stations in the fall in accordance with the SOP MDNR-ESP-113, *Flow Measurements in Open Channels* (MoDNR 2019f). In the spring, stream flow was measured using a Marsh-McBirney Model 2000 Flo-Mate electromagnetic current meter in accordance with SOP MDNR-ESP-113.

4.4 Quality Assurance/Quality Control (QA/QC)

4.4.1 Field Meters

All field meters used to collect water quality parameters were maintained in accordance with *Quality Control Procedures for Checking Water Quality Field Instruments* (MoDNR 2016).

4.4.2 Biological Samples

Steps to assure accuracy of organism removal from sample debris were performed consistent with those methods found in the SMSBPP document (MoDNR 2019c).

4.4.3 Biological Data Entry

All macroinvertebrate data were entered into the WQMS macroinvertebrate database consistent with *Quality Control Procedures for Data Processing* (MoDNR 2019i).

4.4.4 Duplicate Sample Collection

Duplicate samples are obtained from the same location at essentially the same time as the true sample following the same procedures in accordance with *Quality Assurance/Quality Control for Environmental Data Collection* (MoDNR 2019h). True samples and duplicate samples are analyzed for the same set of parameters. A duplicate is used to assess the precision associated with sampling methodology, sample heterogeneity, and analytical procedures (MoDNR 2019h).

A duplicate macroinvertebrate sample was collected at Tributary to Cedar Creek 0.3 during the fall of 2019, and a duplicate benthic sediment sample was collected in May of 2020 at Chat Creek 1.1. No duplicate physicochemical samples were collected during this study.

5.0 Results

5.1 Land Use

The land use data in Table 1 provide a comparison between the 12-digit Hydrologic Unit Code (HUC) of the Douger Branch/Chat Creek study reach, the HWREF streams, and the Ozark/Neosho EDU. All Douger Branch/Chat Creek stations are located within the same 12-digit HUC.

Table 1. Percent Land Use in the Ozark/Neosho EDU, Douger Branch/Chat Creek, and Headwater Reference Stations

	12-digit HUC	Urban/ Impervious	Crop- land	Grass- land	Forest- land	Herba- ceous	Wetland /Open water
Ozark/Neosho EDU	--	5.3	15.2	52.8	20.4	4.8	1.6
Douger Br./Chat Cr.	110702070101	10.9	1.0	74.3	10.5	3.1	0.2
Dry Fork	110702070106	2.5	6.7	79.3	8.0	2.6	1.0
Pottershop Hollow	110702080304	1.4	4.4	56.7	31.3	5.5	0.7
Trib. Cedar Cr.	110702070804	3.9	4.3	59.9	25.9	4.6	1.3
Trib. Glendale Fk.	110702070308	1.8	55.0	31.9	6.5	1.9	2.9
Trib. N. Fk. Spring R.	110702070202	2.6	51.7	35.1	6.1	1.8	2.8

Comparison of land use between the 12-digit HUCs containing the study stream and the Ozark/Neosho EDU showed the study stream watershed contained more urban/impervious land and more grassland than the overall EDU and less cropland and forestland. The HWREF streams varied within each land use category when compared to the study stream and to the EDU.

5.2 Stream Habitat Assessment

Habitat assessment scoring results are found in Table 2. If the study station SHAPP score is ≥ 75 percent of the control station score, the stations are considered to contain comparable habitats to the control station (MoDNR 2019j). Comparable habitats should support similar biological communities. Douger Branch 1.9 scored ≥ 75 percent of the average of HWREF SHAPP scores. Based on SHAPP scores, the instream habitats of Douger Branch 1.9 are similar to the HWREF streams and should, therefore, support a comparable biological community. Douger Branch 0.7 scored 70 percent and Chat Creek 1.1 scored 62 percent when compared to the average of the HWREF SHAPP scores. Based on these SHAPP scores, the stream habitat at these two test stations is considered capable of partially supporting a comparable biological community. It is inferred that these two stations lack comparable habitat to the HWREF streams, and the lack of habitat may affect the biological community.

Table 2. Douger Branch/Chat Creek and Headwater Reference Streams
Habitat Values and Scores

Stream Habitat Parameters	Douger Br 0.7	Douger Br 1.9	Chat Cr 1.1	Dry Fork 2.3	Trib Cedar 0.2	Trib Cedar 0.3
Habitat Assessment Date	10/6/21	10/6/21	10/6/21	9/28/21	9/29/21	9/29/21
Epifaunal Substrate/Avail. Cover	I(18)	I(16)	II(11)	I(17)	I(19)	I(18)
Embeddedness	II(11)	I(18)	II(11)	I(17)	I(17)	I(16)
Velocity/Depth Regime	IV(5)	I(16)	III(9)	III(10)	I(18)	I(18)
Sediment Deposition	II(12)	II(12)	III(6)	II(13)	II(14)	I(16)
Channel Flow Status	III(8)	III(8)	I(18)	III(9)	II(11)	II(13)
Channel Alteration	I(20)	II(15)	III(6)	I(20)	I(20)	I(20)
Riffle Quality	II(12)	II(13)	IV(0)	II(11)	II(12)	II(15)
Left Bank Stability	III(3)	I(9)	II(6)	II(8)	I(10)	I(10)
Right Bank Stability	III(4)	I(10)	III(4)	I(9)	I(9)	II(6)
Left Bank Vegetative Protection	IV(1)	III(5)	IV(1)	IV(1)	III(5)	III(5)
Right Bank Vegetative Protection	IV(1)	III(5)	IV(1)	IV(1)	III(3)	IV(2)
Left Bank Riparian Zone Width	IV(0)	IV(0)	I(10)	I(10)	II(8)	II(8)
Right Bank Riparian Zone Width	II(7)	IV(0)	I(8)	I(10)	IV(2)	II(7)
Total Habitat Score	102	127	91	136	148	154
Percent of the average HWREF Score	70	87	62	Range 136 - 151 Average 146		

Habitat parameter categories range from I to IV with category I = optimal, category II = suboptimal, category III = marginal, and category IV = poor. Habitat parameter scores are listed in parentheses and range from 0 to 20 except for bank stability, vegetative protection, and riparian zone categories, which range from 0 to 10.

Epifaunal substrate in Douger Branch attained an optimal score at both station 0.7 and station 1.9, consisting of a mix of cobble and gravel with some submerged debris present. Embeddedness scored higher at station 0.7 than station 1.9. Douger Branch 0.7 was dominated by a single velocity/depth regime. Station 0.7 was surrounded by cattle pastures and had no channel alterations. The riparian area consisted of few trees along the banks, and vegetative protection of the streambank was low, which contributed to poor bank stability. All four velocity/depth regimes were present at Douger Branch 1.9. Station 1.9 was located downstream of a county road crossing and had little to no riparian area, but vines and grass growing on the streambank offered protection from bank erosion. There were areas of bank stabilization work along the sample reach as well as rip rap at the bridge and near an area where railroad tracks paralleled the stream. Sediment deposition and riffle quality was suboptimal at both Douger Branch stations. Due to the low water levels, channel flow status was marginal.

Chat Creek had a narrow channel with incised banks and lacked flow during fall 2021 (Chat Creek also lacked flow during the fall 2019 bioassessment period) that affected several habitat

assessment parameters. Although Chat Creek lacked flowing water, habitat was evaluated based on the riffle areas that were sampled in spring 2021. Chat Creek had marginal epifaunal substrate consisting of limited areas of gravel. Embeddedness and sediment deposition scored marginally. Chat Creek lacked areas with fast flowing water and had only slow velocity regimes. Although there was no flow during the assessment, channel flow status scored optimal due to the incised nature of the narrow stream bed. Chat Creek is located in a wooded area upstream of the Carnation Road/Hwy 39 bridge. The riparian zone width at this station was optimal with full growth trees and underbrush; invasive honeysuckle was prevalent in the riparian area. Although the banks were incised, the roots of trees and underbrush offered some protection of the streambank.

The HWREF stations had a mix of epifaunal substrate. Tributary to Cedar Creek had larger substrate, and Dry Fork had more woody debris present instream. Embeddedness was optimal at all HWREF stations, with less than 25 percent of the substrate surrounded by fine material. Tributary to Cedar Creek consisted of all four velocity/depth regimes. Dry Fork lacked fast velocity regime areas. Channel flow status was suboptimal at Tributary to Cedar Creek but ranked as marginal at Dry Fork. Riffle quality was suboptimal at all HWREF station, with riffles being as wide as the stream but lacking in length. The HWREF stations showed no evidence of channel alterations. Bank stability varied and scored in the optimal or suboptimal category at each station. Vegetative protection of banks was low, but riparian habitat was generally optimal. The exception was that the riparian zone for Tributary to Cedar Creek 0.2 scored low due to adjacent grassland areas.

5.3 Biological Assessment and Macroinvertebrate Community Analysis

The SMSBPP metric evaluation used numeric criteria that were calculated from two sources. The first source was BIOREF criteria for wadeable/perennial streams (Tables 3 and 4), and the second was from HWREF streams (Tables 5 and 6) within the Ozark/Neosho EDU. Because Douger Branch/Chat Creek is smaller than streams used to calculate BIOREF criteria, five HWREF streams were selected using methods described in Section 2.0. These streams were sampled since larger streams may have more available habitat and higher macroinvertebrate taxa richness than smaller streams.

5.3.1 Comparison with Wadeable/Perennial Biological Criteria Reference Streams

Tables 3 and 4 provide scoring criteria and results for the fall and spring index periods. MSCI scores were calculated by scoring test station biological metrics using BIOREF numeric criteria. An MSCI score of 16-20 is considered fully supporting, 10-14 is partially supporting, and 4-8 is considered non-supporting of the designated beneficial use.

Table 3. Fall 2019 Metric Values and Scores for Douger Branch/Chat Creek
Using Ozark/Neosho Biological Criteria

Stations	TR	EPTT	BI	SDI	MSCI	Support
Douger Branch 0.7	--	--	--	--	--	--
Douger Branch 1.9	48 ₍₃₎	6 ₍₁₎	7 ₍₃₎	2.64 ₍₃₎	10	P
Chat Creek 1.1	--	--	--	--	--	--
Metric Score=5	>77	>24	<5.5	>2.97	20-16	Full
Metric Score=3	77-39	24-12	5.5-7.7	2.97-1.49	14-10	Partial
Metric Score=1	<39	<12	>7.7	<1.49	8-4	Non

MSCI Scoring Table (in light gray) developed from BIOREF samples ($n = 10$). TR = Taxa Richness; EPTT = Ephemeroptera, Plecoptera, Trichoptera Taxa; BI = Biotic Index; SDI = Shannon Diversity Index; (#) subscript indicates individual metric scores.

During the fall of 2019, Douger Branch 0.7 and Chat Creek 1.1 lacked flowing water and consisted only of pooled areas. Due to the lack of sufficient flowing water, bioassessments were not conducted at these two stations. Douger Branch 1.9 had sufficient flowing water and was sampled. Douger Branch 1.9 attained a partially supporting MSCI score of 10 when compared with BIOREF criteria due to low TR and SDI values and a high BI value. The station had 6 EPTT, resulting in a non-supporting score of 1 for this metric.

Table 4. Spring 2021 Metric Values and Scores for Douger Branch/Chat Creek
Using Ozark/Neosho Biological Criteria

Stations	TR	EPTT	BI	SDI	MSCI	Support
Douger Branch 0.7	53 ₍₃₎	3 ₍₁₎	7.1 ₍₃₎	2.94 ₍₃₎	10	P
Douger Branch 1.9	46 ₍₃₎	3 ₍₁₎	6.8 ₍₃₎	2.85 ₍₃₎	10	P
Chat Creek 1.1	28 ₍₁₎	0 ₍₁₎	8.7 ₍₁₎	1.89 ₍₃₎	6	N
Metric Score=5	>72	>27	<5.3	>3.01	20-16	Full
Metric Score=3	72-36	27-13	5.3-7.7	3.01-1.51	14-10	Partial
Metric Score=1	<36	<13	>7.7	<1.51	8-4	Non

MSCI Scoring Table (in light gray) developed from BIOREF samples ($n = 12$). TR = Taxa Richness; EPTT = Ephemeroptera, Plecoptera, Trichoptera Taxa; BI = Biotic Index; SDI = Shannon Diversity Index; (#) subscript indicates individual metric scores.

During spring 2021, both Douger Branch stations attained partially supporting MSCI scores. Both stations had 3 EPTT, resulting in non-supporting scores of 1 for this metric. The remaining three metrics had partially supporting scores. Chat Creek 1.1 scored in the non-supporting MSCI category with non-supporting scores for TR, EPTT, and BI. Chat Creek lacked EPTT entirely.

5.3.2 Comparison with Metrics Calculated from Headwater Reference Streams

Tables 5 and 6 provide scoring criteria calculated from HWREF streams for the fall and spring index periods. MSCI scores were calculated by scoring Douger Branch/Chat Creek biological metrics using metric threshold values calculated from the HWREF stream criteria. An MSCI

score of 16-20 is considered fully supporting, 10-14 is partially supporting, and 4-8 is considered non-supporting of the designated use criteria.

Table 5. Fall 2019 Metric Values and Scores for Douger Branch/Chat Creek
 Using Headwater Reference Stream Criteria

Stations	TR	EPTT	BI	SDI	ΔMSCI	ΔSupport
Douger Branch 0.7	--	--	--	--	--	--
Douger Branch 1.9	48 ₍₃₎	6 _(1→3)	7 _(3→5)	2.64 _(3→5)	10→16	P→F
Chat Creek 1.1	--	--	--	--	--	--
Metric Score=5	>57	>9	<7.1	>2.57	20-16	Full
Metric Score=3	57-29	9-5	7.1-8.5	2.57-1.29	14-10	Partial
Metric Score=1	<29	<5	>8.5	<1.29	8-4	Non

MSCI Scoring Table (in light gray) developed from HWREF streams ($n = 11$). TR = Taxa Richness; EPTT = Ephemeroptera, Plecoptera, Trichoptera Taxa; BI = Biotic Index; SDI = Shannon Diversity Index; (#) subscript indicates individual metric scores. ΔMSCI shows change from BIOREF score to HWREF score. ΔSupport shows change in biological support categories.

During the fall of 2019, Douger Branch 1.9 was the only station sampled. TR and EPTT were below the fully supporting threshold value at this station, resulting in partially supporting scores of 3 for these metrics. BI and SDI attained fully supporting scores of 5 when compared to HWREF criteria. The comparison with similarly sized streams resulted in an MSCI score of fully supporting during fall 2019.

Table 6. Spring 2021 Metric Values and Scores for Douger Branch/Chat Creek
 Using Headwater Reference Stream Criteria

Stations	TR	EPTT	BI	SDI	ΔMSCI	ΔSupport
Douger Branch 0.7	53 ₍₃₎	3 _(1→3)	7.1 ₍₃₎	2.94 _(3→5)	10→14	P _(NC)
Douger Branch 1.9	46 ₍₃₎	3 _(1→3)	6.8 ₍₃₎	2.85 _(3→5)	10→14	P _(NC)
Chat Creek 1.1	28 _(1→3)	0 ₍₁₎	8.7 ₍₁₎	1.89 ₍₃₎	6→8	N _(NC)
Metric Score=5	>53	>7	<6.7	>2.19	20-16	Full
Metric Score=3	53-27	7-3	6.7-8.3	2.19-1.09	14-10	Partial
Metric Score=1	<27	<3	>8.3	<1.09	8-4	Non

MSCI Scoring Table (in light gray) developed from HWREF streams ($n = 14$). TR = Taxa Richness; EPTT = Ephemeroptera, Plecoptera, Trichoptera Taxa; BI = Biotic Index; SDI = Shannon Diversity Index; (#) subscript indicates individual metric scores. ΔMSCI shows change from BIOREF score to HWREF score. ΔSupport shows change in biological support categories. (NC) = no change.

Both Douger Branch stations had partially supporting scores in spring 2021. Little variation existed between the two Douger Branch stations. TR, EPTT, and BI attained scores of 3, and the SDI score increased from 3 to 5 when using HWREF criteria. However, these differences did not change the support category. Chat Creek had non-supporting scores in both the EPTT and the BI metrics, resulting in a non-supporting MSCI score of 8.

Differences existed between BIOREF and HWREF biological metric scoring thresholds during both sampling seasons (Table 7). In the fall, changes to metric scores using HWREF criteria resulted in Douger Branch 1.9 attaining a score of 16, the lowest possible fully supporting MSCI score. Although the threshold values for all four metrics changed, the lower threshold value of EPTT and SDI, combined with the higher threshold value for BI, resulted in a higher MSCI score during the fall. During the spring, the differences between the BIOREF and HWREF resulted in scoring differences for the individual metrics, but these differences did not change the support categories for any of the study stations. Douger Branch 0.7 and 1.9 maintained the partially supporting category, and Chat Creek 1.1 maintained the non-supporting category.

Table 7. BIOREF and HWREF Metric Threshold Comparison

	TR	EPTT	BI	SDI	MSCI	Support
Fall Metric Threshold Values						
BIOREF Metric Score=5	>77	>24	<5.5	>2.97	20-16	Full
BIOREF Metric Score=3	77-39	24-12	5.5-7.7	2.97-1.49	14-10	Partial
BIOREF Metric Score=1	<39	<12	>7.7	<1.49	8-4	Non
HWREF Metric Score=5	>57	>9	<7.1	>2.57	20-16	Full
HWREF Metric Score=3	57-29	9-5	7.1-8.5	2.57-1.29	14-10	Partial
HWREF Metric Score=1	<29	<5	>8.5	<1.29	8-4	Non
Spring Metric Threshold Values						
BIOREF Metric Score=5	>72	>27	<5.3	>3.01	20-16	Full
BIOREF Metric Score=3	72-36	27-13	5.3-7.7	3.01-1.51	14-10	Partial
BIOREF Metric Score=1	<36	<13	>7.7	<1.51	8-4	Non
HWREF Metric Score=5	>53	>7	<6.7	>2.29	20-16	Full
HWREF Metric Score=3	53-27	7-3	6.7-8.5	2.29-1.09	14-10	Partial
HWREF Metric Score=1	<27	<3	>8.3	<1.09	8-4	Non

5.3.3 Macroinvertebrate Community Composition and Analysis

Fall Bioassessment Community Composition and Analysis

Table 8 presents macroinvertebrate community attributes for the fall samples. Figure 4 depicts the percent taxa by BI range, and Figure 5 depicts the percent of taxa by the primary functional feeding group (FFG).

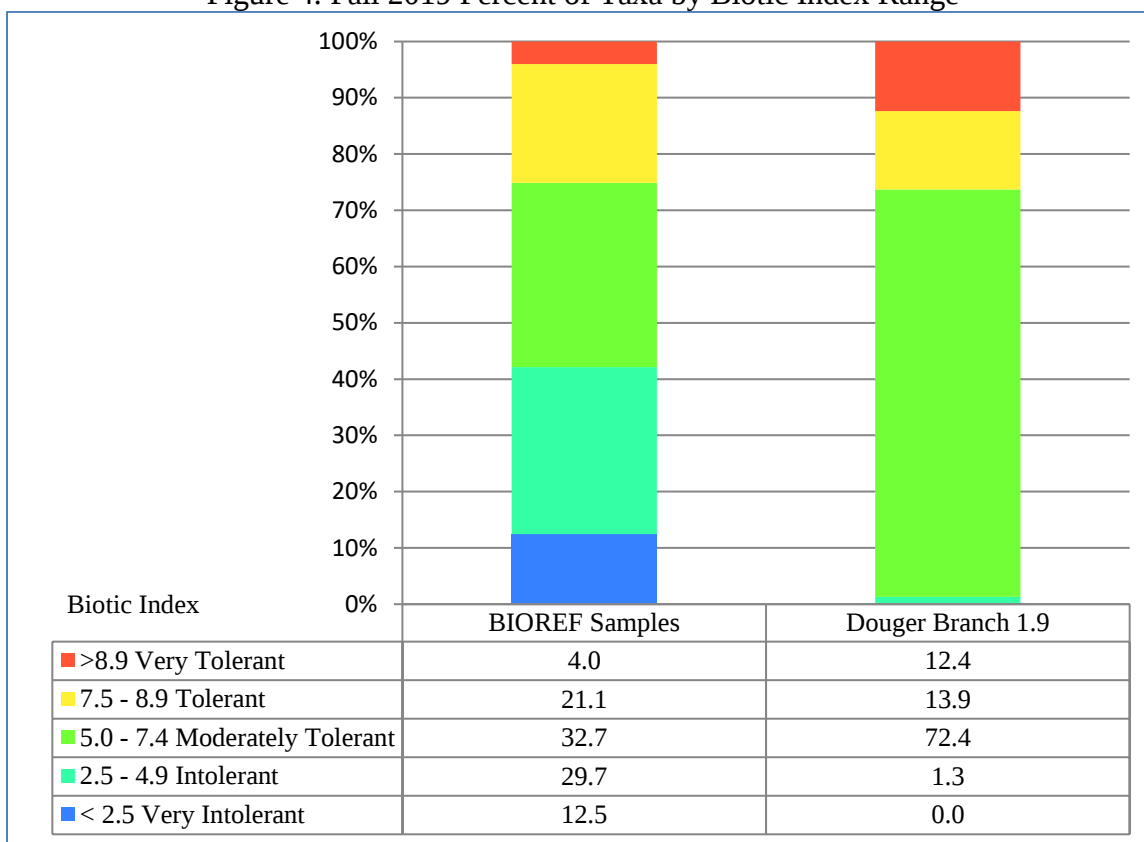
Table 8. Fall 2019 Macroinvertebrate Community Analysis

Stations	BIOREF	Douger	Douger	Chat Creek
Variables	Samples	Branch 0.7	Branch 1.9	1.1
% Ephemeroptera	23.9	--	1.5	--
% Plecoptera	2.0	--	0.0	--
% Trichoptera	11.5	--	27.0	--
% Total EPT	37.4	--	28.5	--
% Diptera	13.6	--	43.2	--
% Dominant Macroinvertebrate Families				
Chironomidae	12.9	--	41.7	--
Hydropsychidae	5.3	--	25.6	--
Elmidae	7.5	--	8.5	--
Physidae	0.2	--	7.5	--
Coenagrionidae	1.1	--	7.0	--
Calopterygidae	0.3	--	2.0	--
Baetidae	4.7	--	1.2	--
Ancylidae	0.6	--	1.2	--

Top 3 families of study station in bold

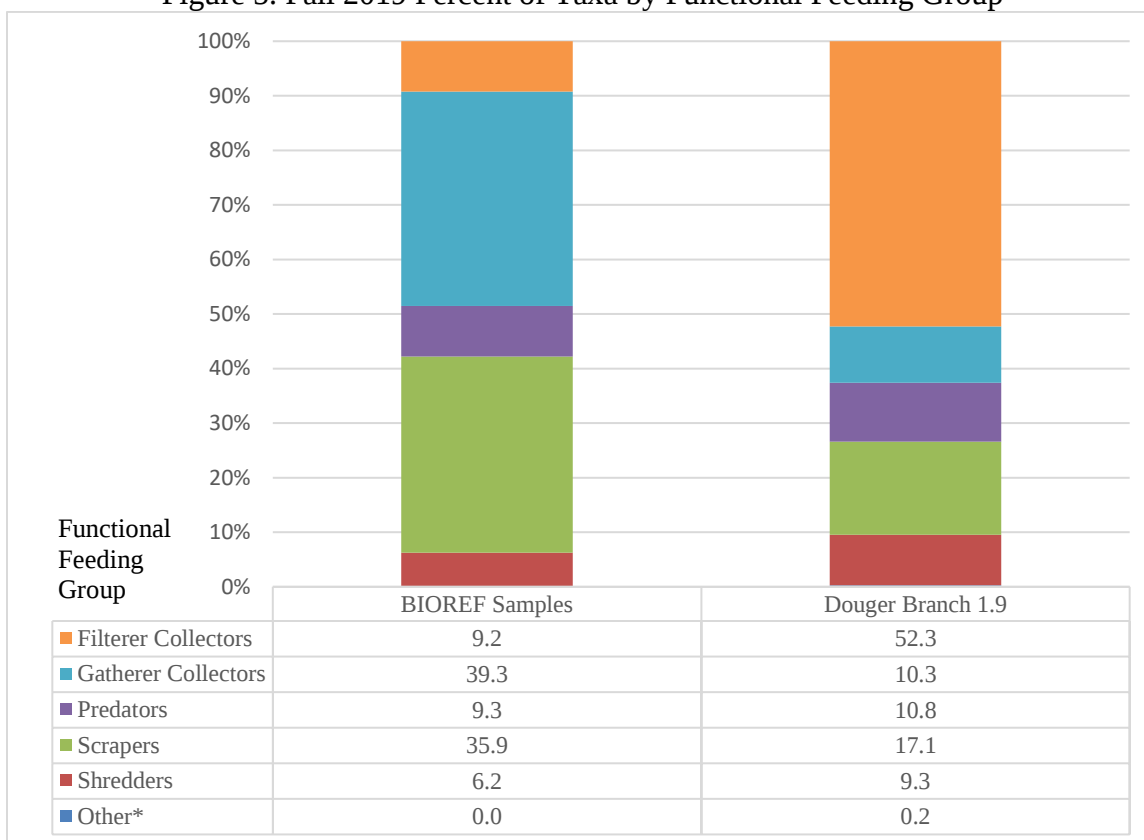
EPT taxa made up 28.5 percent of the Douger Branch 1.9 fall sample (Table 8), of which 25.6 was *Cheumatopsyche* (BI = 6.6). *Baetis* (BI = 6.0) and *Caenis latipennis* (BI = 7.6) were the only mayflies present in Douger Branch. Although Plecoptera were absent from Douger Branch, they were also rarely collected from BIORREF streams during fall sampling. Chironomidae was the most common family, making up almost 42 percent of the community. Hydropsychidae, consisting of the genus *Cheumatopsyche* (BI = 6.6), was the second most common family. Other caddisflies present in Douger Branch included *Hydroptila* (BI = 6.2), *Triaenodes* (BI = 3.7), and *Polycentropus* (BI = 3.5). Diptera accounted for 43.2 percent of the sample and was made up almost entirely of Chironomidae (non-biting midges) taxa. *Tanytarsus* (BI = 6.7), *Microtendipes* (BI = 6.2), and *Cricotopus/Orthocladius* grp. (BI = 6.5) were the most prevalent Chironomidae.

Figure 4. Fall 2019 Percent of Taxa by Biotic Index Range



In fall, 42.2 percent of BIOREF samples were made up of macroinvertebrate taxa in the intolerant BI range (BI < 5.0) (Figure 4). Douger Branch lacked taxa in the very intolerant range (BI < 2.5), and only 1.3 percent of the sample was in the intolerant range (BI=2.5-4.9). Taxa in the more tolerant ranges (BI ≥ 5.0) accounted for 57.8 percent of the taxa at BIOREF stations and 98.7 percent of the taxa at Douger Branch. Of the more tolerant taxa, the very tolerant range (BI ≥ 8.9) was three times higher in Douger Branch than BIOREF samples. Taxa identified in Douger Branch were more tolerant of organic pollution compared to those in BIOREF streams.

Figure 5. Fall 2019 Percent of Taxa by Functional Feeding Group



* Other includes parasites, macrophyte piercers, and taxa with no identified FFG

Gatherer-collectors and scrapers were the most abundant FFG categories among BIOREF streams (Figure 5). Combined, the two categories made up 75.2 percent of the macroinvertebrate community at BIOREF stations. Filterer-collectors, predators, and shredders were more evenly distributed in BIOREF samples. At Douger Branch, gatherer-collectors plus scrapers made up 27.4 percent of the community, and filterer-collectors were the most abundant FFG, accounting for 52.3 percent of the community. Predators were more similar in abundance at Douger Branch.

Spring Bioassessment Community Composition and Analysis

Table 9 presents macroinvertebrate community attributes for the spring sampling season. Figure 6 depicts the percent taxa by BI ranges, and Figure 7 depicts the percent of taxa by the primary FFG.

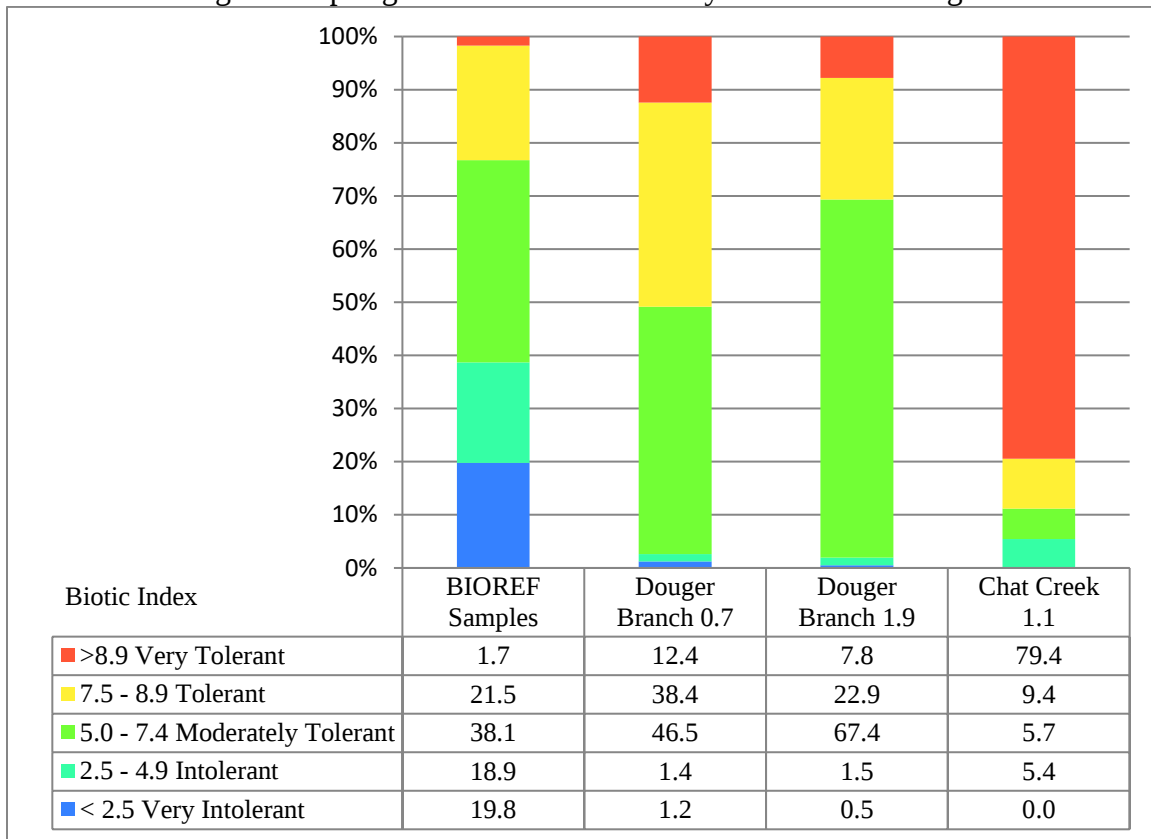
Table 9. Spring 2021 Macroinvertebrate Community Analysis

Stations	BIOREF Samples	Douger Branch 0.7	Douger Branch 1.9	Chat Creek 1.1
Variables				
% Ephemeroptera	27.5	0.0	0.0	0.0
% Plecoptera	7.4	0.0	0.0	0.0
% Trichoptera	4.8	1.0	8.0	0.0
% Total EPT	39.7	1.0	8.0	0.0
% Diptera	30.1	68.2	69.4	7.7
% Dominant Macroinvertebrate Families				
Chironomidae	28.6	67.2	69.2	3.1
Planariidae	0.8	7.5	4.3	6.3
Crangonyctidae	1.3	6.9	1.4	0.3
Elmidae	3.7	5.7	5.7	1.1
Physidae	0.7	0.6	0.6	9.4
Naididae	1.0	1.5	0.2	58.7
Coenagrionidae	0.1	1.3	5.5	0.0
Enchytraeidae	0.1	0.1	1.6	0.6
Hydropsychidae	0.1	0.1	7.2	0.0
Lumbricina	0.1	0.5	0.8	4.0

Top 3 families of study stations in bold

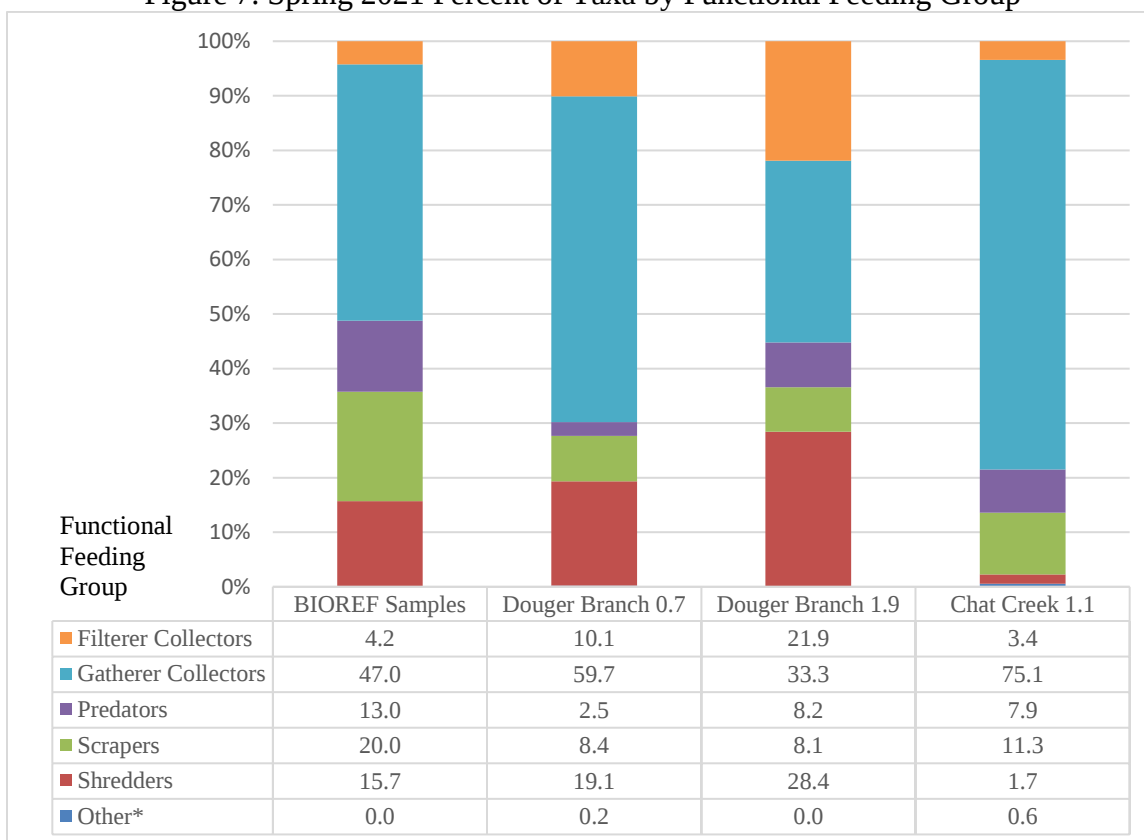
During spring 2021, the three study stations had no Ephemeroptera or Plecoptera taxa. Chat Creek also lacked Trichoptera taxa. Trichoptera were present in the two Douger Branch stations, making up between 0.1 percent to 8.0 percent of samples. *Cheumatopsyche* (BI = 6.6), *Polycentropus* (BI = 3.5), and *Ochrotrichia* (BI = 6.4) were present at Douger Branch 0.7 in low abundance. *Cheumatopsyche* (BI = 6.6), *Triaenodes* (BI = 3.7), and *Polycentropus* (BI = 3.5) were present at Douger Branch 1.9. *Cheumatopsyche* made up 89 percent of the Trichoptera taxa at Douger Branch 1.9. Chironomidae comprised almost 70 percent of taxa at both Douger Branch stations. *Cricotopus/Orthocladius* grp. (BI = 6.5), *Paratanytarsus* (BI = 7.7), *Tanytarsus* (BI = 6.7), and *Corynoneura* (BI = 6.2) were the most common non-biting midge taxa. Non-insect taxa accounted for the majority of taxa at Chat Creek. Tubificinae worms (BI = 9.2), bladder snails (*Physella* BI = 9.1), and flat worms (Planariidae BI = 7.5) were the three most common taxa in Chat Creek.

Figure 6. Spring 2021 Percent of Taxa by Biotic Index Range



During the spring, 38.7 percent of BIOREF samples were made up of macroinvertebrate taxa in the intolerant BI range ($BI < 5.0$) (Figure 6). Among study stations, percentages in the intolerant BI range varied from 2.0 percent at Douger Branch 1.9 to 5.4 percent at Chat Creek. Chat Creek had no taxa in the very intolerant range ($BI < 2.5$). Only two taxa in the intolerant range ($BI 2.5-4.9$) were present at Chat Creek: the dragonfly *Helocordulia* ($BI = 4.8$) and the chironomid *Smittia* ($BI = 4$). Taxa in the more tolerant category ($BI \geq 5.0$) made up 61.3 percent of taxa at BIOREF stations. At the study stations, the more tolerant category ($BI \geq 5.0$) ranged from 94.6 percent at Chat Creek to 98.0 percent at Douger Branch 1.9. Almost 80 percent of the macroinvertebrate community at Chat Creek were very tolerant ($BI \geq 8.9$) of organic pollution.

Figure 7. Spring 2021 Percent of Taxa by Functional Feeding Group



* Other includes parasites, macrophyte piercers, and taxa with no identified FFG

Similar to the fall samples, gatherer-collectors and scrapers were the most abundant FFG categories among BIOREF streams. Combined, these two FFGs made up 67 percent of the community (Figure 7). The remaining categories ranged from 0 percent “others” to 15.7 percent shredders. In the study stations, gatherer collectors alone ranged from 33.3 percent at Douger Branch 1.9 to 75.1 percent of the community at Chat Creek. At the Douger Branch stations, shredders were the second most common FFG, and at Chat Creek, scrapers were the second most common.

5.4 Physicochemical Water Parameters

Discharge, *in-situ* water quality results, and dissolved metals analysis for the Douger Branch/Chat Creek stations and the HWREF streams are presented in Tables 10 through 15. During the fall of 2019, Douger Branch station 0.7 and Chat Creek 1.1 had no flowing water and were not sampled. Although there are currently no nutrient criteria for Missouri streams and rivers, nutrient parameters were compared to the U.S. EPA’s December 2000 publication *Ambient Water Quality Criteria Recommendations for Rivers and Streams in Nutrient Ecoregion XI* (USEPA 2000). The recommended values for the Ozark Highlands, which includes the study stations and the HWREF stations, are: 0.24 mg/L nitrate+nitrite as N, 0.38 mg/L total nitrogen, 0.007 mg/L total phosphorus, and 1.4 NTU turbidity. EPA’s nutrient criteria targets are not tied

to specific biological condition or Missouri's minimum dissolved oxygen (**DO**) criteria and simply represent an administrative water quality protection policy that guides EPA's clean water programs.

Table 10. Fall 2019 Discharge and *In-Situ* Water Quality Measurements

Parameters	Douger Br. 0.7*	Douger Br. 1.9	Chat Cr. 1.1*	Dry Fork 2.3	Trib. Cedar 0.2	Trib. Cedar 0.3
Sampling time/date	--	1255; 9/24	--	0900; 9/25	1230; 9/25	1435; 9/25
Dissolved Oxygen (mg/L)	--	6.92	--	4.38**	8.72	8.86
pH (su)	--	8.0	--	7.19	7.35	7.24
Conductivity (µS/cm)	--	626	--	476	274	268
Temperature (°C)	--	20.0	--	19.3	18.0	18.2
Turbidity (NTU)	--	1.0	--	<1.0	1.7	3.0
Discharge (cfs)	--	0.9	--	<0.1	9.4	9.1

*Douger Br 0.7 and Chat Cr 1.1 had insufficient flow to adequately sample.

** Value below minimum WQS of 5.0 mg/L.

Values in bold exceed EPA's recommendations.

DO was below the WQS minimum of 5.0 mg/L at Dry Fork, and flow was below 0.1 cfs. DO was above 5.0 mg/L at Douger Branch and the Tributary to Cedar Creek stations. Conductivity was lowest at the Tributary to Cedar stations. Turbidity values exceeded EPA's recommended value at the Tributary to Cedar Creek stations.

Table 11. Fall 2019 Water Chemistry Concentrations

Station	Douger Br. 0.7*	Douger Br. 1.9	Chat Cr.1.1*	Dry Fork 2.3	Trib. Cedar 0.2	Trib. Cedar 0.3
Sampling time/date	--	1255; 9/24	--	0900; 9/25	1230; 9/25	1435; 9/25
Nitrate+Nitrite as N (mg/L)	--	3.99 ⁰⁹	--	16.6 ⁰⁹	3.36 ⁰⁹	3.15 ⁰⁹
Total Nitrogen (mg/L)	--	4.29	--	15.8 ⁰⁹	3.02	2.89
Total Phosphorus (mg/L)	--	10.2 ⁰⁹	--	<0.005 ND	<0.005 ND	<0.005 ND
Ammonia as N (mg/L)	--	<0.02 ND	--	<0.02 ND	0.11	<0.02 ND
Sulfate (mg/L)	--	47.1	--	7.91	3.62 ⁰⁵	3.53 ⁰⁵
Chloride (mg/L)	--	53.7	--	18.0	9.09	8.80
Total Suspended Solids (mg/L)	--	<5 ND	--	5.0	<5 ND	<5 ND

05 estimated value, detected below the practical quantitation limit; 09 sample diluted during analysis; ND not detected at reported value. Values in bold exceed EPA's recommendations.

Nitrate+nitrite as N was elevated at Douger Branch and all the HWREF stations to the extent it had to be diluted during analysis. Total nitrogen was also elevated at all stations but only required dilution at Dry Fork. Total phosphorus was below detectable concentrations at HWREF stations, but it required dilution during analysis at Douger Branch. Sulfate and chloride were lowest at the Tributary to Cedar Creek stations.

Table 12. Fall 2019 Dissolved Metals Concentrations

Station	Douger Br. 0.7	Douger Br. 1.9	Chat Cr. 1.1	Dry Fork 2.3	Trib. Cedar 0.2	Trib. Cedar 0.3
Sampling time/date	--	1255; 9/24	--	0900; 9/25	1230 9/25	1435; 9/25
Barium-Dissolved (µg/L)	--	31.4	--	122	53.3	52.1
Cadmium-Dissolved (µg/L)	--	0.24	--	<0.10 ND	0.22	0.23
Lead-Dissolved (µg/L)	--	<0.5 ND	--	<0.50 ND	<0.5 ND	<0.5 ND
Nickel-Dissolved (µg/L)	--	3.35	--	3.28	2.21	2.16
Zinc-Dissolved (µg/L)	--	35.0	--	0.70 ⁰⁵	22.0	22.1
Calcium-Dissolved (mg/L)	--	50.7	--	86.3	52.6	51.6
Magnesium-Dissolved (mg/L)	--	15.6	--	4.20	2.40	2.37
Hardness as CaCO ₃ (mg/L)	--	191	--	233	141	139

05 estimated value, detected below the practical quantitation limit; ND not detected at reported value

There were no exceedances of dissolved metals at either Douger Branch or the HWREF stations during the fall of 2019.

Table 13. Spring 2021 Discharge and *In-Situ* Water Quality Measurements

Stations	Douger Br. 0.7	Douger Br. 1.9	Chat Cr. 1.1	Dry Fork 2.3	Trib. Cedar 0.2	Trib. Cedar 0.3
Parameters						
Sampling time/date	1026; 3/22	1145; 3/22	1250; 3/22	1047; 3/29	1230; 3/29	1345; 3/29
Dissolved Oxygen (mg/L)	10.96	11.63	12.04	11.75	11.14	10.83
pH (su)	8.01	8.02	8.10	7.98	7.78	7.75
Conductivity (µS/cm)	366	388	439	382	258	263
Temperature (°C)	11.0	11.7	11.7	10.3	15.1	15.7
Turbidity (NTU)	1.76	1.70	1.60	1.70	3.33	2.53
Discharge (cfs)	17.9	14.2	2.5	3.6	16.5	17.9

Values in bold exceed EPA's recommendations.

During the spring of 2021, dissolved oxygen was similar among the study stream stations and the HWREF stations. Conductivity was highest at the Chat Creek station and lowest at the Tributary to Cedar Creek stations. Temperature was highest at the Tributary to Cedar Creek stations. Turbidity exceeded EPA's recommendations at all stations.

Table 14. Spring 2021 Water Chemistry Concentrations

Stations	Douger Br. 0.7	Douger Br. 1.9	Chat Cr. 1.1	Dry Fork 2.3	Trib. Cedar 0.2	Trib. Cedar 0.3
Sampling time/date	1026; 3/22	1145; 3/22	1250; 3/22	1047; 3/29	1230; 3/29	1345; 3/29
Nitrate+Nitrite as N (mg/L)	3.39 ⁰⁹	3.07 ⁰⁹	0.52	15.6 ⁰⁹	2.30 ⁰⁹	2.30 ⁰⁹
Total Nitrogen (mg/L)	3.54	3.10	0.58	14.7 ⁰⁹	2.24	2.36
Total Phosphorus (mg/L)	0.97	0.88	0.07	0.08	0.07	0.08
Ammonia as N (mg/L)	<0.02 ND	0.3 ⁰⁵	<0.02 ND	<0.02 ND	<0.02 ND	<0.02 ND
Sulfate (mg/L)	21.3	23.0	40.0	9.64	6.65	4.68 ⁰⁵
Chloride (mg/L)	15.8	16.0	9.05	14.0	7.54	7.75
Total Suspended Solids (mg/L)	<5 ND	<5 ND	<5 ND	<5 ND	<5 ND	<5 ND

05 Estimated value, result between method detection limit and practical quantitation limit; 09 sample diluted during analysis; ND not detected at reported value. Values in bold exceed EPA's recommendations.

Similar to the fall, nitrate+nitrite as N exceeded EPA's recommended value of 0.24 mg/L for the Ozark Highlands at all stations. At Douger Branch and the HWREF stations, nitrate+nitrite as N had to be diluted for analysis. At Chat Creek, nitrate+nitrite as N was considerably lower compared to the other stations sampled. Total nitrogen was higher than EPA's recommended value of 0.38 mg/L at all stations but only required dilution at Dry Fork; again, this was a pattern similar to fall samples. Total phosphorus exceeded EPA's recommended value of 0.007 mg/L at all stations. Sulfate and chloride were lowest at the Tributary to Cedar Creek stations.

Table 15. Spring 2021 Dissolved Metals Concentrations

Stations	Douger Br. 0.7	Douger Br. 1.9	Chat Cr. 1.1	Dry Fork 2.3	Trib. Cedar 0.2	Trib. Cedar 0.3
Sampling time/date	1026; 3/22	1145; 3/22	1250; 3/22	1047; 3/29	1230; 3/29	1345; 3/29
Barium-Dissolved (µg/L)	52.1	55.3	79.4	88.3	46.1	46.6
Cadmium-Dissolved (µg/L)	0.10 ⁰⁵	0.11 ⁰⁵	0.40	<0.1 ND	0.18 ⁰⁵	0.19 ⁰⁵
Lead-Dissolved (µg/L)	<0.5 ND	<0.5 ND	<0.5 ND	<0.5 ND	<0.5 ND	<0.5 ND
Nickel-Dissolved (µg/L)	1.01	1.07	1.41	<0.5 ND	<0.5 ND	<0.5 ND
Zinc-Dissolved (µg/L)	64.4	76.7	314	<0.5 ND	24.2	21.1
Calcium-Dissolved (mg/L)	51.2	55.5	78.3	63.7	46.6	47.1
Magnesium-Dissolved (mg/L)	6.42	6.67	5.95	3.82	2.14	2.13
Hardness as CaCO ₃ (mg/L)	154	166	220	175	125	126

05 Estimated value, result between method detection limit and practical quantitation limit; ND not detected at reported value. Bold value exceeds WQS.

There were no exceedances of dissolved metals at either Douger Branch or the HWREF stations during the fall of 2019. At Chat Creek, zinc exceeded both the acute and chronic criteria (both criteria are 229 µg/L), which are hardness dependent based calculations from Table A2 in the Missouri WQS (MoDNR 2018f).

5.5 Benthic Sediment Analysis for Metals

In May of 2020, benthic sediment samples were collected using a 2 mm (#10) sized sieve at all Douger Branch/Chat Creek stations (Table 16) and three of the HWREF sampling stations (Table 17). A duplicate sample was collected at the Chat Creek station. Individual metals concentrations were compared to PEC recommendations (MacDonald et al. 2000). A PEC is a threshold level for a contaminant, above which harmful effects are likely to be observed. MacDonald et al. (2000) found PECs to be reliable for 10 metals (including cadmium, lead, and zinc) in classifying sediments as potentially toxic or nontoxic to sediment-dwelling organisms. The toxic effects from a mixture or combination of metals may be examined using the sum of PEQs for cadmium, lead, and zinc ($\sum \text{PEQ}_{\text{Cd, Pb, Zn}}$) (MacDonald 2009 et al). Sediment is considered to pose a low risk if the $\sum \text{PEQ}_{\text{Cd, Pb, Zn}} < 7.92$ (MacDonald et al. 2009).

Table 16. Sieved Benthic Sediment Metals Concentrations of
Douger Branch and Chat Creek

Stations	Douger Br. 0.7	Douger Br. 1.9	Chat Cr. 1.1	Chat Cr. 1.1 (dup)	PEC	PEQ Threshold
Sampling time/date	1050; 5/18	1247; 5/18	1208; 5/18	1208; 5/18		
Arsenic mg/kg (PEQ)	30.7 ⁰⁹ (0.93)	21.2 (0.64)	49.8⁰⁹ (1.51)	43.8⁰⁹ (1.33)	33.0	>1.0
Cadmium mg/kg (PEQ)	7.42¹¹ (1.49)	7.74¹¹ (1.55)	24.7¹¹ (4.69)	14.1¹¹ (2.83)	4.98	>1.0
Chromium mg/kg (PEQ)	281.0 (2.53)	270.0 (2.43)	275.0 (2.48)	257.0 (2.32)	111.0	>1.0
Copper mg/kg (PEQ)	18.1 (0.12)	10.8 (0.0.7)	25.9 (0.17)	23.3 (0.16)	149.0	>1.0
Lead mg/kg (PEQ)	281.0 (2.20)	284.0 (2.22)	1,710.0⁰⁹ (13.36)	1,230.0⁰⁹ (9.61)	128.0	>1.0
Nickel mg/kg (PEQ)	55.8⁰⁹ (1.15)	33.4⁰⁹ (0.69)	74.3⁰⁹ (1.53)	52.2⁰⁹ (1.07)	48.6	>1.0
Zinc mg/kg (PEQ)	642.0⁰⁹ (1.40)	1,100.0⁰⁹ (2.40)	4,970.0⁰⁹ (10.83)	3,130.0⁰⁹ (6.82)	459.0	>1.0
TOC mg/kg	1620	5200	3150	7190	--	--
$\sum \text{PEQ}_{\text{Cd, Pb, Zn}}$	5.08	6.17	28.88	19.26	--	>7.92

⁰⁹ sample diluted during analysis; ¹¹ estimated value, matrix interference; TOC = total organic carbon
Bold values are above the PEC and/or PEQ threshold.

Although MoDNR's analysis included a suite of metals, cadmium, lead, and zinc are the three metals of main concern at Douger Branch/Chat Creek. These three metals exceeded the PEC and the PEQ threshold at all sampling stations and were highest at Chat Creek. Cadmium was as much as 5 times the PEC at Chat Creek. Lead was 10-13 times higher than the PEC, and zinc was 7-10 times higher than the PEC in Chat Creek duplicate samples. All three metals were

elevated at the Douger Branch stations as well, but not to the degree observed in Chat Creek. Lead was diluted for analysis at Chat Creek, and zinc was diluted for analysis at all three sampling stations. The sum of the PEQs of cadmium, lead and zinc, which examines the toxic effects from a mixture or combination of these metals, exceeded the threshold of >7.92 at Chat Creek but was below the threshold at Douger Branch.

Arsenic and nickel were diluted for analysis at Douger Branch 0.7 and Chat Creek. Nickel was also diluted for analysis at Douger Branch 1.9. Arsenic, chromium, and nickel also exceeded the PEC and PEQ thresholds at Chat Creek. At Douger Branch, chromium exceeded the PEC and PEQ thresholds at both stations, and nickel exceeded the PEC at the most downstream station.

Table 17. Sieved Benthic Sediment Metals Concentrations
of Headwater Reference Streams

Stations	Dry Fork 2.3	Trib. Cedar 0.2	Trib. Cedar 0.3	PEC	PEQ Threshold
Sampling time/date	1040; 5/20	1230; 5/20	1255; 5/20	--	--
Arsenic mg/kg (PEQ)	38.8 ⁰⁹	4.98	10.6	33.0	>1.0
	(1.18)	(0.15)	(0.32)		
Cadmium mg/kg (PEQ)	6.9 ¹¹	4.64 ¹¹	4.18 ¹¹	4.98	>1.0
	(1.39)	(0.93)	(0.84)		
Chromium mg/kg (PEQ)	158.0	56.9	93.6	111.0	>1.0
	(1.42)	(0.51)	(0.84)		
Copper mg/kg (PEQ)	14.3	5.54	3.55	149.0	>1.0
	(0.10)	(0.04)	(0.02)		
Lead mg/kg (PEQ)	55.4	218.0	259.0	128.0	>1.0
	(0.43)	(1.70)	(2.02)		
Nickel mg/kg (PEQ)	49.7 ⁰⁹	9.52	10.3	48.6	>1.0
	(1.02)	(0.20)	(0.21)		
Zinc mg/kg (PEQ)	73.6	335.0	335.0	459.0	>1.0
	(0.16)	(0.73)	(0.73)		
TOC mg/kg	2220	32400	3460	--	--
ΣPEQ _{Cd, Pb, Zn}	1.98	3.36	3.59	--	>7.92

09 sample diluted during analysis; 11 estimated value, matrix interference; TOC = total organic carbon.
Bold values are above the PEC and/or PEQ threshold.

At Dry Fork, arsenic, cadmium, chromium, and nickel were above the PEC and the PEQ thresholds. Arsenic and nickel were diluted for analysis at Dry Fork. At both of the Tributary to Cedar Creek stations, lead was the only metal in exceedance of the PEC and PEQ thresholds. The sum of cadmium, lead, and zinc did not exceed the threshold of >7.92 at the HWREF stations (Table 17).

5.6 Quality Assurance/Quality Control (QA/QC)

Quantitative Similarity Index for Taxa (QSIT) is a sampling protocol quality control measure that expresses taxa similarity between two samples as a percentage. QSIT was calculated for the duplicate macroinvertebrate samples collected at Tributary to Cedar Creek during the fall of 2019. Duplicate samples are expected to have a score of 70 percent or greater (Rabeni et al. 1999, MoDNR 2019c). During the fall of 2019, the QSIT at Tributary to Cedar Creek was 83.8.

The duplicate sample difference criteria for the physicochemical and benthic sediment parameters are specified in the Fiscal Year 2019 Quality Assurance Project Plan (MoDNR 2019b). No duplicate physicochemical samples were collected at the study stations or the HWREF stations during this study. A duplicate sample was collected for benthic sediment analysis during May of 2020 at Chat Creek 1.1. Cadmium, lead, nickel, and zinc exceeded the 20 percent relative percent difference duplicate sample criterion. This difference is likely the result of the heterogeneity of the benthic sediment.

6.0 Discussion

6.1 Land Use

The study stream originates just southeast of the city limits of Aurora and flows directly through the city to its confluence with Spring Creek. Throughout the city, the stream is mostly a maintained ditch. The downstream section of the creek flows through agricultural lands comprising cropland and cattle pastures.

Urbanization affects the quantity and quality of water entering a watershed by altering the natural flow of rainwater, thus making streams and rivers more susceptible to extreme flooding events (Tang et al. 2005, Walsh et al. 2005). Impervious surfaces increase the amount of stormwater, which often contains sediment and pollutants that have accumulated in runoff coming from paved streets and parking lots, rooftops, manicured lawns, etc. (Lenat and Crawford 1994, Paul and Meyer 2001, Walsh et al. 2005). Urban/impervious surfaces account for 10.9 percent of the land use in the Douger Branch/Chat Creek watershed, twice as much urban/impervious surface compared to BIOREF streams in the EDU. The urbanization in the study streams' watershed is confined to the headwaters (Figure 3). The study streams' watershed only contains 1 percent cropland but grassland (pasture) makes up almost 75 percent of the watershed. The most downstream station was located in a cattle pasture in which livestock had direct access to the stream. However, it should be noted that the stream does not maintain permanent flow due the nature of being a losing stream. Streams in urban and agricultural areas tend to have increased concentrations of sediment, nutrients, and contaminants as well as macroinvertebrate communities with reduced biotic richness and increased dominance of tolerant taxa (Allan 2004, Walsh et al. 2005).

The Tri-State Mining District of Kansas, Missouri, and Oklahoma was once one of the major producers of lead and zinc in the world. Lead and zinc mining has historically occurred in the Baldwin Park area, located in northeast Aurora. Mining ceased in the mid-1950s, and the underground mines filled with groundwater. Zinc-contaminated groundwater has been resurfacing into a small tributary of Douger Branch/Chat Creek (locally called Baldwin Park

Tributary) (MoDNR 2006). Zinc is an essential nutrient to aquatic and terrestrial organisms, but in excess can be highly toxic and has the tendency to bioaccumulate in the environment. Baldwin Park was first investigated by EPA in 1979 because a portion of the park was used as a dump site following closure of the mines in the area. Combined efforts of sampling from 1979 onward by EPA, the MoDNR Water Protection Program (WPP), MoDNR Division of Geology and Land Survey (DGLS), and the MoDNR Hazardous Waste Program (HWP) concluded that mine waste contamination was present (MoDNR 2006).

6.2 Stream Habitat Assessment

Instream habitat variables as well as catchment variables have been shown to influence aquatic communities (Wang et al. 2007, Burdon et al. 2013). Epifaunal substrate varied among stations, but sites lacked larger substrate such as cobble and contained minimal woody debris. Riffles were of poor to marginal quality, and the study stream lacked pool variability. Chat Creek had incised banks and a narrow channel. A study on macroinvertebrate conditions throughout the Corn Belt of the United States showed instream stressors were dominant factors driving macroinvertebrate communities (Waite and Van Metre 2017). Burdon et al. (2013) determined sensitive taxa are more strongly associated with benthic habitat than with food resources. Lack of benthic habitat therefore limits sensitive taxa.

A study of agricultural streams in New Zealand found that a decrease of instream habitat availability through sedimentation decreased macroinvertebrate biodiversity and that sensitive taxa are strongly associated with benthic habitat quality (Burdon et al. 2013). Although this study did not specifically examine sedimentation, all stations were affected by sedimentation. At Chat Creek, sedimentation affected nearly 50 percent of the stream's substrate. Sedimentation homogenizes the stream bed by filling interstitial spaces of the substrate and voids that would otherwise be created with instream debris. This decreases the available benthic habitat.

Intact riparian zones along stream banks are important in maintaining stream health, stream habitat, and water quality (Allan 2004, Waite and Van Metre 2017). Douger Branch stations had sparse vegetative protection. Much of the stream length is surrounded by cattle pastures with sparse riparian areas. Although the Chat Creek station had a well-established riparian zone, the stream upstream of the study station was surrounded by urban areas with a mix of residential, commercial, and industrial properties. The stream begins just outside of the city limits on the southeast side of Aurora and mostly flows as ditches that entirely lack a riparian corridor through the city. Overall, the entire stream reach has limited riparian areas. Riparian vegetation positively influences stream ecosystems directly by shading and litter inputs, and indirectly by reducing inputs of nutrients and other contaminants entering the system from the surrounding landscape (Burrell et al 2013).

6.3 Biological Assessment and Macroinvertebrate Community Analyses

Comparison of EDU and Headwater References

Differences existed when assessing Douger Branch/Chat Creek using BIOREF versus HWREF biological criteria. Threshold values for each of the biological metrics used to calculate MSCI scores were affected when compared to the HWREF criteria for both sampling seasons. During

the fall, Douger Branch 1.9, the only station sampled, changed from partially supporting to fully supporting when using HWREF criteria. The lower thresholds for EPTT and SDI, combined with the higher BI threshold, elevated the station to an MSCI score of 16, the lowest possible fully supporting score. Although MSCI scores increased when using HWREF criteria for each station sampled in spring, there were no changes in support category. The lower thresholds for EPTT and SDI when compared to the HWREF criteria elevated both Douger Branch stations from MSCI scores of 10 to scores of 14, which is still within the partially supporting category. The lower TR threshold increased the Chat Creek station MSCI score from 6 to 8, but this station remained in the non-supporting category. Even with the lower thresholds for each category contained in the MSCI score, the study stream still failed to attain fully supporting status.

Percent Composition of Sensitive Taxa

Based on the comparison of percent sensitive taxa and the overall macroinvertebrate community composition, the macroinvertebrate community of Douger Branch/Chat Creek was dominated by moderately tolerant (BI range 5.0-7.4) to tolerant (BI range 7.5-8.9) taxa. Taxa with high BI values are often associated with organic and nutrient pollution (Hilsenhoff 1987). The study stream consistently had low numbers of sensitive taxa. During the fall, mayflies were scarce, and stoneflies were lacking entirely (stoneflies were rarely collected at the BIOREF stations as well). *Cheumatopsyche* (BI = 6.6) was the dominant caddisfly. During the spring, all study stations lacked mayflies and stoneflies. Caddisflies were collected at the Douger Branch stations but were rare at the most downstream Douger Branch station. Caddisflies made up 8.0 percent of the community at Douger Branch 1.9, the only station to maintain flowing water throughout both sampling seasons. Chat Creek lacked caddisflies and was dominated by very tolerant taxa (BI range >8.9).

Effects from urban areas and WWTFs have been shown to contribute to declines in aquatic communities and biological integrity. Runoff from impervious surfaces and point source discharges results in contaminants and excessive nutrient influxes into waterways, causing taxa richness declines in aquatic communities (Paul and Meyer 2001). Poulton and Tao (2019) showed a decline in EPT abundance and richness at sites affected by urbanization and point sources. The study stream suffers extensively from urbanization. Upstream, the stream is simply a ditch running through the city. The two Douger Branch stations, both of which are affected by urbanization and the WWTF, had similar MSCI scores during the spring (the only assessment period in which both stations were sampled). Lenat and Crawford (1994) found that streams in urban settings suffer from low taxa richness, low abundance, and high biotic index values.

Percent Composition of Functional Feeding Groups

Because FFGs respond to inputs into aquatic systems, stressed aquatic conditions are often manifested in FFG categories. Specialist FFG categories have specific feeding needs that may be limited in impaired systems, whereas generalist FFGs are thought to be more tolerant due to their ability to use a wider array of available food resources. Generalist categories, such as gatherer-collectors and filterer-collectors, increase in response to increased pollution or disturbances while specialists such as scrapers decrease (Barbour et al. 1999). Filterer-collectors dominated the aquatic community at Douger Branch 1.9 during the fall. During the spring, gatherer-

collectors dominated the aquatic community at the study stations. During both seasons, the community of scrapers at the study stations was half of what was present at the BIOREF stations.

6.4 Physicochemical Water Parameters

One notable trend in water chemistry was the elevated nutrient concentrations. High levels of nutrients result in degraded aquatic communities (Waite and Van Metre 2017, Wang et al. 2007). Douger Branch 1.9, the only study station sampled during the fall, had elevated levels of nitrate+nitrite as N, total nitrogen, and total phosphorus. During spring sampling, both Douger Branch stations had elevated nutrients, and nutrients were lowest in Chat Creek. The stations on Douger Branch are located downstream of the WWTF. Due to the low discharge in the stream, there was less stream flow available for wastewater dilution. Additionally, the stream is classified as a losing stream; therefore, the majority of the flow is likely attributable to the discharge from the facility. Dyer and Wang (2002) reported declines in EPTT, and Suckling (1982) observed increases in hydropsychid caddisflies downstream of WWTF outfalls. Fall samples contained moderately tolerant mayflies, lacked stoneflies, and caddisflies consisted mostly of the family Hydropsychidae. Spring samples lacked mayflies and stoneflies and, similar to fall samples, the caddisflies present were mostly hydropsychids.

Dry Fork was higher in nitrate+nitrite as N and total nitrogen compared to the study stations and the other two HWREF stations during both bioassessment periods. Although the cause of the high nutrient values in Dry Fork is unknown, much of the land use upstream of the sampling point is agricultural land, and there are permitted concentrated animal feeding operations (poultry) in neighboring watersheds. It is unknown whether there are smaller unpermitted animal feeding operations present in the Dry Fork watershed; however, it is possible poultry litter is land applied within the watershed.

All study stations and the HWREF stations are located in the Ozark Highlands subregion specified in EPA's December 2000 *Ambient Water Quality Criteria Recommendations for Rivers and Streams in Nutrient Region XI* (USEPA 2000). Although these EPA targets are not used in a regulatory setting, they do represent guidelines for EPA's clean water programs. Nitrate+nitrite as N was as much as 70 times higher than EPA's recommended 0.24 mg/L at Dry Fork and 16 times higher than the recommended target at Douger Branch. Total nitrogen was 40 times higher than EPA's recommended target of 0.38 mg/L at Dry Fork and 11 times higher at Douger Branch. Total phosphorus, which was diluted for analysis at Douger Branch, was 10.2 mg/L during the fall, almost 1,500 times EPA's recommended target value of 0.007 mg/L for the Ozark Highlands subregion. Excessive nutrients can potentially threaten aquatic communities by causing algal blooms and eutrophication. Excessive nutrients have been identified as a major reason why water quality objectives, such as support of aquatic life, fail (USEPA 2000).

Missouri's WQS establish acute and chronic numeric criteria for dissolved metals for aquatic life protection. Missouri's WQS Table A1 refers to Table A2 for both acute and chronic numeric criteria. Table A2 displays criteria equations for metals that are hardness dependent. There were no exceedances of dissolved metals during the fall sampling period. During the spring, zinc exceeded the WQS acute (229 µg/L) and chronic (229 µg/L) criteria at Chat Creek by 31 percent.

Chapter 10 CSR 20-7.031(5)(B)1 of Missouri's WQS specifies that the numeric criteria found in Tables A1, A2, B2, and B3 of the WQS shall not be exceeded. Any exceedances, therefore, are a violation of Missouri's WQS. Aquatic organisms are exposed to dissolved metals through absorption to or uptake across the gills. Metals in water and in sediment can affect aquatic organisms (Rainbow 1996, Maret et al. 2003).

6.5 Benthic Sediment Analysis Results

Mining operations were present in the study area between the 1870s and 1950s. Although mining operations ceased in the 1950s, mine waste continues to be a source of heavy metals contamination in the watershed, specifically of cadmium, lead, and zinc. Johnson et al. (2016) found that the highest concentrations of metals contamination are associated with geographical locations of mining. Increased levels of metals contamination in soils and water columns in mining districts can accumulate and persist within the environment. Surface water runoff through mining tailings piles and emergence of groundwater from abandoned mines transports the contaminated soils and water throughout the watershed. Concentrations of metals in benthic stream sediments in sufficient amounts that result in toxicity to aquatic life or that harm benthic organisms violate the general criteria of Missouri's Water Quality Standards at 10 CSR 20-7.031(4)(D) and (5)(B)1. Per 10 CSR 20-7.031(4)(D), "waters shall be free from substances or conditions in sufficient amounts to result in toxicity to human, animal, or aquatic life."

Cadmium, lead, and zinc exceeded the PEC and the PEQ at all study stations. At the most upstream station, Chat Creek 1.1, cadmium was approximately 5 times higher than the PEC, and lead and zinc were roughly 10 times higher than the PEC. MacDonald et al. (2009) considered the combined effects from cadmium, lead, and zinc pose a low risk if the sum of the PEQ of the three metals ($\sum \text{PEQ}_{\text{Cd, Pb, Zn}}$) is less than 7.92. At Chat Creek, the sum of these metals was 28.88. Metal uptake through diet is a critical pathway of accumulation in aquatic biota. The complexities in feeding strategies and diet make estimates of the true harm of dissolved metals difficult (Cain et al. 2011). Detrimental effects of long term exposure to metals in aquatic biota (e.g. decreased richness, reduced abundance, composition shifts) have been well documented (Clements et al. 1992, Clements et al. 2000, Brumbaugh et al. 2005, Allert et al. 2012, Besser et al. 2015). In addition to the biomagnification potential throughout the food chain, decreased biodiversity and population sizes affect both aquatic as well as terrestrial systems.

Benthic sediment analysis revealed exceedances of metals at the HWREF streams. At Dry Fork, arsenic, cadmium, chromium, and nickel exceeded the PEC and the PEQ thresholds. Lead exceeded the PEC and PEQ thresholds at both of the Tributary to Cedar Creek stations. These exceedances were less severe than exceedances of the study stations. The sum of the PEQ of cadmium, lead, and zinc was below 7.92 at all HWREF stations. Minimal mining occurred in the watersheds of the HWREF streams, so it is unknown why the benthic sediment metals were elevated at Dry Fork and the Tributary to Cedar Creek stations.

6.6 Water Quality Duplicate Sample Results

The duplicate benthic sediment analysis exceeded the 20 percent relative percent difference (RPD) for the following parameters: cadmium (RPD 55 percent), lead (RPD 33 percent), nickel (RPD 35 percent), and zinc (RPD 45 percent). Review of collection and analysis did not reveal any apparent reason for the differences. The differences are likely due to heterogeneity of the benthic sediment.

7.0 Summary

Seven null hypotheses were stated in the introduction.

- 1) Land cover characteristics in the Douger Branch/Chat Creek watershed will be similar to land cover characteristics of the Ozark/Neosho EDU. This hypothesis is rejected. The land use comparison showed differences between the 12-digit HUC containing the Douger Branch/Chat Creek study stations and the Ozark/Neosho EDU. The study stream contained less cropland and forestland but more grassland and urban/impervious land than the EDU.
- 2) Stream habitat assessment scores of Douger Branch/Chat Creek will be similar to the average stream habitat scores of the HWREF streams located within the Ozark/Neosho EDU. This hypothesis is rejected for two stations, Douger Branch 0.7 and Chat Creek 1.1. Douger Branch 1.9 was the only station to score >75 percent of the average of the HWREF stream habitat score.
- 3) The macroinvertebrate community in Douger Branch/Chat Creek will be similar among longitudinally separate reaches of Douger Branch/Chat Creek. This hypothesis is rejected. Differences among the stations were evident during the spring bioassessment, the only season with enough stream flow to sample all study stations. The two Douger Branch stations had similar community attributes and scored similarly during the spring, but the Chat Creek station differed.
- 4) The macroinvertebrate community in Douger Branch/Chat Creek will be similar to the macroinvertebrate community of BIOREF streams in the Ozark/Neosho EDU. This hypothesis is rejected. MSCI scores were partially supporting or non-supporting during both seasons.
- 5) The macroinvertebrate community in Douger Branch/Chat Creek will be similar to the macroinvertebrate community of HWREF streams in the Ozark/Neosho EDU. This hypothesis is accepted for the fall sampling period but rejected in the spring sampling period. During the fall 2019 bioassessment period, Douger Branch scored in the fully supporting category, but the sampling stations scored partially to non-supporting when compared to HWREF criteria in spring 2021.
- 6) Physicochemical water quality of the study stream will meet the WQS and U.S. EPA's recommended nutrient levels. This hypothesis is partially accepted. Dissolved zinc exceeded the chronic and acute WQS at Chat Creek. Nutrients were elevated at the study stations during both sampling seasons when compared with U.S. EPA's recommended values. The remaining water chemistry values were within the WQS criteria limits.

7) Metals toxicity values of benthic sediment samples collected from Douger Branch/Chat Creek will meet recommended PEC values calculated by MacDonald et al. (2000), and the values will be below the PEQ threshold. This hypothesis is rejected. The only metal to not be in exceedance of the PEC and/or the PEQ was copper. Arsenic was below the PEC and PEQ at Douger Branch but exceeded the thresholds at Chat Creek. Nickel was below the PEC and PEQ only at Douger Branch 1.9.

8.0 Conclusion

Stormwater runoff from mine waste piles as well as contaminated groundwater within abandoned mines are subject to elevated metals loading. Mine waste continues to cause metals contamination in the watershed. Other mining related activities also contribute to the metals contamination in the region. Mine tailings are often reprocessed, resulting in fine-grained materials that are more prone to erosional and transport processes. These fine particles can have high concentrations of cadmium, lead, and zinc (Schneider et al. 2007). Processed tailings also have been used as aggregate for road construction and fill activities throughout the region, including backfill for sewage collection lines.

Douger Branch/Chat Creek is classified as a losing stream, losing a significant amount of water into the groundwater system. This same groundwater system, contaminated from past mining activities, recharges the stream. The upstream portion of the stream is urbanized and maintained as a ditch with little to no flow. Although the WWTF provides a continuous flow of water to the stream, Douger Branch station 1.9 was the only station to maintain enough flow to conduct bioassessments during both the fall and spring period. Douger Branch 0.7 was pooled with large expanses of gravel bars between pooled areas. Even though station 0.7 is a little over 1 mile downstream of station 1.9, the discharge from the facility is lost into the subsurface before reaching the downstream station. The aquatic community is affected by the lack of permanent flow.

Compared to reference conditions from the Ozark/Neosho EDU, the study stream had a higher percentage of urban land cover and grassland (pastures). When comparing the study stations to HWREF streams of similar size, the stations did not consistently attain the optimal MSCI scores necessary to indicate full support of the protection of warm water habitat beneficial use designation. The entire stream reach exhibited signs of degraded conditions. The stream lacked flow and contained few EPT taxa. Land use features led to a macroinvertebrate community composed primarily of moderately tolerant or tolerant taxa. The macroinvertebrate community may be suffering from long term effects of landscape alterations. It may only take a fragment of time to alter the surrounding landscape; however, it may take much longer for the macroinvertebrate community to recover from long term sustained effects of anthropogenic disturbances (Harding et al. 1998).

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Appendix A

Fall and Spring Metric Values and Scores for Headwater Reference streams
Using Ozark/Neosho Biological Criteria

Fall Metric Values and Scores for Headwater Reference Streams
Using Ozark/Neosho Biological Criteria

Stations	Year	TR	EPTT	BI	SDI	MSCI	Support
Trib. Cedar Cr.	2017	62 ₍₃₎	21 ₍₃₎	6.1 ₍₃₎	2.65 ₍₃₎	12	P
Trib. Cedar Cr.	2017	64 ₍₃₎	16 ₍₃₎	6.7 ₍₃₎	2.4 ₍₃₎	12	P
Pottershop Hollow	2017	82 ₍₅₎	19 ₍₃₎	5.7 ₍₃₎	3.25 ₍₅₎	16	F
Trib. NF Spring R.	2017	54 ₍₃₎	2 ₍₁₎	7.9 ₍₁₎	3.01 ₍₅₎	10	P
Trib. Glendale Fk.	2017	53 ₍₃₎	5 ₍₁₎	7.5 ₍₃₎	2.6 ₍₃₎	10	P
Trib. Cedar Cr.	2018	68 ₍₃₎	20 ₍₃₎	6.1 ₍₃₎	2.9 ₍₃₎	12	P
Trib. Glendale Fk.	2018	53 ₍₃₎	3 ₍₁₎	8.4 ₍₁₎	2.48 ₍₃₎	8	N
Trib. Cedar Cr.	2019	79 ₍₅₎	20 ₍₃₎	6.1 ₍₃₎	2.94 ₍₃₎	14	P
Trib. Cedar Cr.	2019	59 ₍₃₎	18 ₍₃₎	6.3 ₍₃₎	2.78 ₍₃₎	12	P
Trib. Cedar Cr.	2019	63 ₍₃₎	15 ₍₃₎	6.3 ₍₃₎	2.95 ₍₃₎	12	P
Dry Fk.	2019	70 ₍₃₎	13 ₍₃₎	6 ₍₃₎	3.05 ₍₅₎	14	P
BIOREF Score=5	---	>77	>24	<5.5	>2.97	20-16	Full
BIOREF Score=3	---	77-39	24-12	5.5-7.7	2.97-1.49	14-10	Partial
BIOREF Score=1	---	<39	<12	>7.7	<1.49	8-4	Non

MSCI Scoring Table (in light gray) developed from BIORREF streams ($n = 10$). TR = Taxa Richness; EPTT = Ephemeroptera, Plecoptera, Trichoptera Taxa; BI = Biotic Index; SDI = Shannon Diversity Index; Individual metric scores in parentheses.

Spring Metric Values and Scores for Headwater Reference Streams
Using Ozark/Neosho Biological Criteria

Stations	Year	TR	EPTT	BI	SDI	MSCI	Support
Trib. NF Spring R	2018	44 ₍₃₎	1 ₍₁₎	7.7 ₍₃₎	2.67 ₍₃₎	10	P
Trib. Glendale Fk.	2018	54 ₍₃₎	6 ₍₁₎	6.7 ₍₃₎	2.76 ₍₃₎	10	P
Pottershop Hollow	2018	79 ₍₅₎	22 ₍₃₎	5.4 ₍₃₎	3.27 ₍₅₎	16	F
Trib. Cedar Cr.	2018	61 ₍₃₎	20 ₍₃₎	6.1 ₍₃₎	2.44 ₍₃₎	12	P
Trib. Cedar Cr.	2018	63 ₍₃₎	18 ₍₃₎	6.2 ₍₃₎	2.4 ₍₃₎	12	P
Trib. Cedar Cr.	2018	32 ₍₁₎	4 ₍₁₎	6.7 ₍₃₎	2.17 ₍₃₎	8	N
Pottershop Hollow	2019	64 ₍₃₎	19 ₍₃₎	4 ₍₅₎	2.66 ₍₃₎	14	P
Trib. Cedar Cr	2019	33 ₍₁₎	11 ₍₁₎	7.2 ₍₃₎	1.81 ₍₃₎	8	N
Trib. Cedar Cr.	2019	67 ₍₃₎	19 ₍₃₎	6.1 ₍₃₎	2.43 ₍₃₎	12	P
Trib. Cedar Cr.	2019	63 ₍₃₎	20 ₍₃₎	6.3 ₍₃₎	2.46 ₍₃₎	12	P
Trib. Glendale Fk.	2019	52 ₍₃₎	2 ₍₁₎	7.7 ₍₃₎	2.36 ₍₃₎	10	P
Dry Fk.	2021	56 ₍₃₎	16 ₍₃₎	6 ₍₃₎	2.25 ₍₃₎	12	P
Trib. Cedar Cr.	2021	68 ₍₃₎	21 ₍₃₎	6.4 ₍₃₎	1.94 ₍₃₎	12	P
Trib. Cedar Cr.	2021	69 ₍₃₎	25 ₍₃₎	6.6 ₍₃₎	1.91 ₍₃₎	12	P
BIOREF Score=5	---	>72	>27	<5.3	>3.01	20-16	Full
BIOREF Score=3	---	72-36	27-13	5.3-7.7	3.01-1.51	14-10	Partial
BIOREF Score=1	---	<36	<13	>7.7	<1.51	8-4	Non

MSCI Scoring Table (in light gray) developed from BIORREF streams ($n = 12$). TR = Taxa Richness; EPTT = Ephemeroptera, Plecoptera, Trichoptera Taxa; BI = Biotic Index; SDI = Shannon Diversity Index; Individual metric scores in parentheses.

Appendix B

Invertebrate Database Bench Sheet Report
Fall 2019 and Spring 2021

Douger Branch and Chat Creek
Grouped by Season and Station

Douger Branch 1.9, Lawrence County			
September 24, 2019			
* - large rare			
ORDER: Observed Taxa	CS	NF	RM
AMPHIPODA			
Stygobromus		1	
BASOMMATOPHORA			
Ancylidae		11	3
Helisoma		1	
Physella	7	6	77
COLEOPTERA			
Dubiraphia		2	4
Optioservus sandersoni	1		
Psephenus herricki	1		
Scirtidae			1
Stenelmis	3	39	53
DECAPODA			
Faxonius neglectus	2	*	5
Faxonius virilis		*	
DIPTERA			
Ablabesmyia	2	10	1
Ceratopogoninae			1
Chironomus		15	
Cricotopus bicinctus	10		3
Cricotopus/Orthocladius	51	3	7
Cryptochironomus		1	
Dicrotendipes	1	25	10
Ephydridae	1	1	
Forcipomyiinae			2
Microtendipes	2	76	5
Paratanytarsus	3	6	6
Paratendipes		5	
Phaenopsectra		2	1
Polypedilum flavum	3		
Polypedilum illinoense grp	4		
Polypedilum scalaenum grp		5	
Rheotanytarsus	15		
Stempellinella			1
Tabanus	*		
Tanytarsus	134	55	34
Thienemanniella	2		

Douger Branch 1.9, Lawrence County			
Thienemannimyia grp	5		
Tipula	13	*	
EPHEMEROPTERA			
Baetis	14		
Caenis latipennis		3	1
HEMIPTERA			
Rhagovelia	1		
LUMBRICINA			
Lumbricina	*	7	
ODONATA			
Argia	21	16	15
Calopteryx			21
Enallagma			32
Hetaerina			3
TRICHOPTERA			
Cheumatopsyche	308		
Hydroptila	3		
Polycentropus	1		
Triaenodes			13
TRICLADIDA			
Planariidae	6	1	
VENEROIDA			
Pisidiidae			1

Douger Branch 0.7, Lawrence County			
March 22, 2021			
* - large rare			
ORDER: Observed Taxa	CS	NF	RM
AMPHIPODA			
Stygobromus	2	64	
ARHYNCHOBDSELLIDA			
Erpobdellidae	1		
BASOMMATOPHORA			
Ancylidae		1	
Lymnaeidae			1
Physella	1		5
COLEOPTERA			
Dubiraphia	1		1
Dytiscidae		3	1
Neoporus		1	
Optioservus sandersoni	1		
Peltodytes		1	
Stenelmis	16	32	4
DECAPODA			
Faxonius neglectus	2	1	1
DIPTERA			
Ablabesmyia	2		
Corynoneura	50	30	29
Cricotopus bicinctus			1
Cricotopus/Orthocladius	77	16	21
Diamesa	7	2	1
Dicrotendipes	13	27	26
Diplocladius	1		
Ephydriidae			2
Gonomyia		1	
Hydrobaenus		3	1
Limnophyes	1	5	1
Micropsectra	3	4	4
Microtendipes		1	
Muscidae			1
Parametriocnemus	2		
Paraphaenocladius	1		1
Paratanytarsus	8	26	80
Paratendipes	2	16	2
Phaenopsectra	3	6	6

Douger Branch 0.7, Lawrence County			
Polypedilum flavum	1		
Polypedilum illinoense grp	19	5	42
Pseudosmittia			1
Rheotanytarsus	3	2	6
Simulium	4		
Smittia		2	
Tanytarsus	15	30	29
Thienemanniella	4		5
Thienemannimyia grp	1		
Tipula	*		1
ISOPODA			
Caecidotea (Blind & Unpigmented)		43	
LUMBRICINA			
Lumbricina		5	
ODONATA			
Argia		4	3
Calopteryx			1
Enallagma			5
TRICHOPTERA			
Cheumatopsyche	4		3
Ochrotrichia			1
Polycentropus	1		
TRICLADIDA			
Planariidae	40	15	17
TUBIFICIDA			
Enchytraeidae	6	2	1
Limnodrilus hoffmeisteri	1	1	
Tubificinae		11	1

Douger Branch 1.9, Lawrence County			
March 22, 2021			
* - large rare			
ORDER: Observed Taxa	CS	NF	RM
AMPHIPODA			
Stygobromus		17	
ARHYNCHOBDSELLIDA			
Erpobdellidae		1	
BASOMMATOPHORA			
Ancylidae	1	1	

Douger Branch 1.9, Lawrence County			
Helisoma		1	
Physella		1	6
BRANCHIOBDELLIDA			
Branchiobdellida		1	
COLEOPTERA			
Dubiraphia			1
Stenelmis	24	38	4
DECAPODA			
Faxonius neglectus	1	3	4
DIPTERA			
Ablabesmyia		6	
Chironomidae	2	1	1
Corynoneura	23	18	51
Cricotopus bicinctus	2		
Cricotopus/Orthocladius	196	28	24
Dicrotendipes	10	22	12
Micropsectra	2	1	3
Microtendipes	1	2	
Muscidae	1		
Myxosargus	1	1	
Parametriocnemus	1		
Paraphaenocladius	1	1	2
Paratanytarsus	9	28	45
Paratendipes	2	25	2
Phaenopsectra	4	9	7
Polypedilum aviceps		1	
Polypedilum flavum	43	2	
Polypedilum illinoense grp	11	4	14
Polypedilum scalaenum grp	2	4	
Rheotanytarsus	6		4
Tanytarsus	69	68	19
Thienemanniella	7		3
Thienemannimyia grp	4	2	2
Tipula	*		
LUMBRICINA			
Lumbricina	1	9	
ODONATA			
Aeshnidae			1
Argia	31	3	9
Calopteryx			9

Douger Branch 1.9, Lawrence County			
Enallagma			22
TRICHOPTERA			
Cheumatopsyche	84		1
Polycentropus	2	3	
Triaenodes			4
TRICLADIDA			
Planariidae	46	3	2
TUBIFICIDA			
Enchytraeidae	3	5	11
Limnodrilus hoffmeisteri		1	
Tubificinae			1
VENEROIDA			
Pisidiidae	2	2	

Chat Creek 1.1, Lawrence County			
March 22, 2021			
* - large rare			
ORDER: Observed Taxa	CS	NF	RM
AMPHIPODA			
Stygobromus		1	
ARHYNCHOBDELLIDA			
Erpobdellidae		1	*
BASOMMATOPHORA			
Gyraulius			1
Lymnaeidae	1		
Menetus		1	
Physella	1	4	28
COLEOPTERA			
Dytiscidae	2	4	6
Stenelmis	2	1	1
DIPTERA			
Caloparyphus			1
Ceratopogoninae	1		
Chironomus		1	
Cricotopus/Orthocladius	1		
Diamesa	1		
Diptera		2	
Myxosargus	3	3	1
Paratendipes	1	1	
Smittia	2	3	1

Chat Creek 1.1, Lawrence County			
Tipula		5	
ISOPODA			
Caecidotea (Blind & Unpigmented)			1
LUMBRICINA			
Lumbricina	11	3	
ODONATA			
Helocordulia		1	12
Pachydiplax longipennis		1	
TRICLADIDA			
Planariidae	5	8	9
TUBIFICIDA			
Enchytraeidae	1	1	
Limnodrilus claparedianus		7	
Limnodrilus hoffmeisteri	1	5	
Tubificinae	4	187	3
VENEROIDA			
Pisidiidae	1	9	2